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GUANIDINYL AND RELATED CELL ADHESION MODULATION COMPOUNDS

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Guanidinyl-containing compounds, and related compounds and salts, useful as cell receptor antagonists for modulating cell adhesion via integrin receptors and/or fibronectin receptors, are disclosed. Methods for synthesizing, testing, formulating, and using the compounds as therapeutic agents are also disclosed.

GUANIDINYL AND RELATED CELL ADHESION
MODULATION COMPOUNDS

BACKGROUND OF THE INVENTION

1.   Field of the Invention

5   The present invention relates to novel guanidinyl and related compounds which are characterized by cell adhesion modulation activity.   The compounds have application to the study and treatment of disease conditions mediated by cell adhesion.   Specifically, the compounds have application to the study, diagnosis, treatment or prevention of diseases and conditions such as, for example,

10   cardiovascular disease, harmful platelet aggregation, neoplastic disease including metastasis of neoplastic growth, wound healing, inflammation and autoimmune disease or other diseases or conditions involving cell adhesion.

2.   Related Art

The extracellular matrix is that material which surrounds the muscle and is the major component of connective tissue of all mammals. The extracellular matrix provides for structural integrity, and promotes cell migration and cellular differentiation. As part of these functions, the extracellular matrix has been shown to support adhesion for various types of cells *in vitro*. Molecules such as the collagen, fibronectin, vitronectin, laminin, von Willebrand factor, thrombospondin, bone sialoprotein, fibrinogen, and tenacin have been found to possess this property of mediating cell adhesion.

The above cell-adhesive molecules have been found to exhibit a structural similarity in their respective binding sites, each of which contains the amino acid sequence arginine-glycine-aspartic acid, or RGD using conventional single letter nomenclature.   The cell-binding site in fibronectin has been reproduced synthetically. In turn, the cellular receptor site for fibronectin has been identified for various cells. In addition, cellular receptors that recognize RGD-containing sequences in other extracellular matrix proteins (e.g., the vitronectin receptor) have been identified.

Such cellular receptors, responsive to RGD-containing proteinaceous compounds, have been characterized.   The complete, primary structure of the fibronectin receptor has been deduced from cDNA and physical properties have been determined. Argraves, *et al., J.Biol. Chem.,* **1986,** 261, 12922; Argraves *et al., J.Biol. Chem.,* **1987,** 105, 1183. The protein exists at the cell surface as a heterodimeric complex (although the larger polypeptide is enzymatically processed) having both polypeptide chains inserted into the membrane. Each chain extends

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30-40 residues into the cytoplasmic space, and at least one of the cytoplasmic peptides appears to interact with the cytoskeleton. Horwitz et al., Nature, 1986, 320, 531. The larger of the two polypeptides, the α subunit, contains a number of regions that are structurally similar to calmodulin and that apparently mediate the binding of calcium to the receptor. The presence of such divalent cations is required for the receptor to bind ligand. The β subunit is somewhat smaller and conformationally compact due to numerous intrachain disulfide bonds. The cytoplasmic domain of the β subunit contains a potentially phosphorylated tyrosine. Hirst et al., PNAS-USA, 1986, 83, 6470; Tamkun et al., Cell, 1986, 46, 271-282.

Other RGD-directed receptors, as well as other "orphan" receptors the ligand for which is unknown, have also been characterized. This putative RGD commonality of the ligand matrix proteins has revealed a superfamily of cell surface receptor proteins that share a high degree of structural similarity and probably also functional similarity. The members of this superfamily of cell surface proteins collectively are known as the integrins. The integrins can be grouped on the basis of the identity of their β subunit. The β subunit, as disclosed above for the fibronectin receptor, is compact due to a high degree of cross-linking. The first group of integrins includes the very late activation antigen (VLA) proteins, which themselves include the fibronectin receptor (VLA-5), the collagen receptor (VLA-2), and the laminin receptor. The second group includes the lymphocyte associated antigen-1 (LFA-1), macrophage antigen-1 (MAC-1), and p150,95. The third group includes the vitronectin receptor, and platelet glycoprotein gpIIb/IIIa. Hynes, Cell, 1987, 48, 549; Hemler, Immunol. Today, 1988, 9, 109; Springer et al., Annu. Rev. Immunol., 1987, 5, 223; Kishimoto et al., Leukocyte Adhesion Molecules, T.A.

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Springer, D.C. Anderson, A.S. Rosenthal, and R. Rothlein, Ed., Springer-Verlag: New York; 1989, pp. 7-43.

The RGD-directed receptor present on platelets that binds fibronectin, vitronectin, fibrinogen, and von Willebrand factor has also been purified. This receptor is the gpIIb/IIIa protein complex. This receptor is thus not specific to one extracellular matrix protein, as are the above fibronectin and vitronectin receptors. It has been proposed that this lack of specificity is correlated to the lack of conformational specificity in the ligands. Other work has suggested that specificity can be achieved with relatively short, conformationally restricted synthetic peptides containing the RGD sequence. For a literature summary, see: Pierschbacher *et al.*, *Nature*, 1984, 309, 30 (1984); Pierschbacher *et al.*, *PNAS-USA*, 1984, 81, 5985; Ruoslahti *et al.*, *Cell*, 1986, 44, 517; Pierschbacher *et al.*, *Science*, 1987, 238, 491; Pierschbacher *et al.*, *J.Biol.Chem.*, 1987, 262, 17294; Hynes, *Cell*, 1987, 48, 549; Ruoslahti, *Ann. Rev. Biochem.*, 1988, 57, 375. It has also been proposed that the receptor affinity for its peptide ligand may be altered as the stereoconformation, or three-dimensional shape, of the peptide is restricted, typically by cyclization. Pierschbacher and Ruoslahti, PCT International Publication WO 89/05150 (1989).

A limited number of compounds containing sequences of natural amino acids or derivatives other than RGD may also possess the capability for affecting cell adhesion. These non-RGD-containing peptides are not well characterized. See, Graf, J. *et al.*, *Cell*, 1987, 48, 989; Kloezewiak, M. *et al.*, *Biochemistry*, 1984, 23, 1767-1774; Wayner, E.A., *et al.*, *J. Cell. Biol.*, 1989, 109, 1321.

U.S. Patent No. 4,879,313 to Tjoeng, *et al.* reports the utility as platelet aggregation inhibitors of certain peptide mimetic compounds containing, in addition to a

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guanidinyl group at one terminus and an internal aspartic acid residue, an aromatic structure (phenyl, biphenyl, naphthyl, pyridyl or thienyl groups, and certain methoxy-substituted forms thereof) at another defined position in the compound. Related structures containing an internal glycine residue are reported in U.S. Patent

5   No. 4,857,508 to Adams, *et al.*

All publications, patents and other reference materials to which reference is made in the present specification are incorporated herein by reference.

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SUMMARY OF THE INVENTION

The present invention relates to compounds having activity as cell adhesion modulators. The compounds are characterized by the presence of a guanidinyl or related group (e.g., substituted guanidinyl, urea or thiourea group) linked through a hydrocarbon moiety, and optionally also through an amino acid residue, to a carboxyl- or carboxyl-derivative-containing portion comprising an aspartic acid-related residue and, optionally, an additional amino acid residue or analog thereof. The carboxyl or carboxyl-derivative portion of the compounds may have either a normal- or a reverse-orientation peptide bond structure, and (especially in the latter case) may contain one or more D-enantiomer residues.

The compounds, in one aspect, sufficiently mimic extracellular matrix ligands or other cell adhesion ligands so as to bind to cell surface receptors. Such receptors include integrin receptors in general, including the fibronectin, collagen, laminin, LFA-1, MAC-1, p150,95, vitronectin and gpIIb/IIIa receptors. The novel compounds have been found to modulate cell adhesion by competing, for example, with RGD-containing ligands and by binding to RGD-directed receptors on cell surfaces. Such cell adhesion ligands, including (but not limited to) fibronectin, are sufficiently inhibited from binding to the cell's receptor as to prevent or reduce cell adhesion. Other uses include enhancing cell adhesion by using the compounds to attach cells to a surface, or by other promotion of cell adhesion. The useful compounds herein described function as cell-adhesion modulators.

One object of the present invention is to provide novel compounds which act to modulate cell adhesion.

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Another object of the present invention is to provide novel guanidinyl and related compounds which are capable of binding with a cellular receptor.

Another object of the present invention is to provide novel guanidinyl and related compounds which contain one or more "reverse" amino acid residues and which are capable of binding to a cellular receptor.

Another object of the present invention is to provide a novel method for modulating cell adhesion using novel compounds.

Another object of the present invention is to provide novel compounds, formulations, and methods which may be used in the study, diagnosis, treatment or prevention of diseases and conditions which relate to cell adhesion, including but not limited to rheumatoid arthritis, asthma, allergies, adult respiratory distress syndrome (ARDS), cardiovascular disease, thrombosis or harmful platelet aggregation, reocclusion following thrombolysis, neoplastic disease including metastasis of neoplastic growth, wound healing, Type I diabetes, inflammatory conditions including ophthalmic inflammatory conditions and inflammatory bowel disease (e.g, ulcerative colitis and regional enteritis), and autoimmune diseases.

Another object is to provide derivative compounds, such as, but not limited to, antibodies and anti-idiotype antibodies to the compounds disclosed and claimed in order to study, diagnose, treat or prevent diseases and conditions which relate to cell adhesion, including but not limited to rheumatoid arthritis, asthma, allergies, adult respiratory distress syndrome (ARDS), cardiovascular disease, thrombosis or harmful platelet aggregation, reocclusion following thrombolysis, neoplastic disease

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including metastasis of neoplastic growth, wound healing, Type I diabetes, inflammatory conditions and autoimmune diseases.

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BRIEF DESCRIPTION OF THE DRAWING

The accompanying figure (FIGURE 1) is a graphical representation showing cell adhesion inhibition by a representative guanidinyl compound of the invention, 8-guanidinyloctanoyl-(O-phenylmethyl)-aspartyl-(O-phenylmethyl)serine phenylmethyl

5   ester.

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DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention are those having the property of modulating cell adhesion.

5   While cell adhesion is required for certain normal physiological functions, situations exist in which cell adhesion is undesirable, or in which modulated cell adhesion is desirable.

Altered leukocyte-endothelial interactions are implicated in adult respiratory distress syndrome (ARDS). Here, the attachment of inappropriate cells to the lung lining induces an inflammatory response. This results in lung injury, ARDS and in some 10   cases, asthma. Preliminary *in vitro* results show that such detrimental attachment, in which the leukocyte adheres to endothelial cells or the lung extracellular matrix, is mediated by RGD-containing protein and RGD-recognizing receptors on the leukocytes. In this situation, peptides or other compounds with a binding affinity to RGD receptors are desirable as competitive antagonists and should be useful 15   in treating ARDS and asthma. Such compounds are disclosed herein.

Cell adhesion also contributes to metastasis of cancerous tumors. Metastasis has been called "the major underlying cause of death from cancer." Welch, *et al.*, *Intern. J. Cancer*, 1989, 43, 449. An RGD-containing peptide which would prevent cell adhesion to basement membrane components may be useful to prevent or 20   eliminate metastasis. See, Humphries, M.J. *et al.*, *Science*, 1986, 233, 467; Liotta, L.A., *Cancer Res.*, 1986, 46, 1; Roose, E., *Biochem. Biophys. Acta.*, 1986, 738, 263. A peptide or other compound with suitable affinity for RGD receptors, such as disclosed herein, should likewise have anti-metastasis utility.

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Harmful blood clotting is also caused by inappropriate cell adhesion, particularly cell adhesion to the extracellular matrix. The attachment, spreading and aggregation of platelets on extracellular matrices are central events in thrombus formation. These events can be regulated by the family of platelet adhesive glycoproteins, fibrinogen, fibronectin, and von Willebrand factor. Fibrinogen functions as a cofactor for platelet aggregation, while fibronectin supports platelet attachment and spreading reactions. Von Willebrand factor is important in platelet attachment to and spreading on subendothelial matrices. Plow *et al.*, *PNAS-USA*, **1985**, **82**, 8057. A peptide or other compound, such as these herein, which would function as an antagonist and bind to cell receptors which recognize the matrix glycoprotein RGD site would be beneficial as a thrombolytic.

Other physiological conditions may be treated by stimulatory modulation of cell adhesion. Wound healing, for example, is undesirably prolonged when insufficient cell adhesion occurs. A peptide or other compound with suitable affinity for RGD receptors, attached for example to a suitably positioned matrix or surface, may be able to promote beneficial cell adhesion and resultant wound healing by binding cells with the appropriate RGD-recognizing receptor. Also, in prosthetic implantation, such peptides or other compounds coating the prosthesis would provide a means for covering the prosthesis with a surface of cells. This cell surface would provide a surface compatible with the organism, and thus minimize rejection that might otherwise occur due to stimulation of the immune system by the prosthesis itself. The compounds of the present invention are believed to be useful in this cell adhesion modulation application as well.

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The cell adhesion modulation compounds of the present invention are represented in part herein by amino acid residues wherein the individual amino acids are represented by their standard three-letter abbreviations as follows:

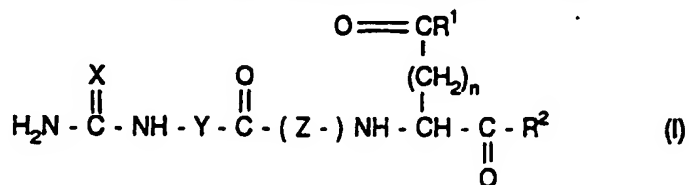| Amino Acid	Three-letter symbol
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Cysteine	Cys
Glutamine	Gln
Glutamic acid	Glu
Glycine	Gly
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr
Valine	Val

Where abbreviations such as the foregoing are used herein without an indication of enantiomeric structure, either the L- or D-enantiomers may suitably be utilized, although the L-enantiomer is preferred for amino acids having the normal (non-reversed) peptide bond orientation, and the D-enantiomer is preferred for amino acids having a reversed orientation (see discussion below).

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Additional abbreviations used herein include:

β-Ala	β-Alanine (3-aminopropionic acid)
BOC	tert-butyloxycarbonyl
Cbz	Benzyloxycarbonyl
DCC	Dicyclohexylcarbodiimide
DCHA	Dicyclohexylamine
DIEA	Diisopropylethylamine
DMF	Dimethylformamide
FMOC	Fluorenylmethyloxycarbonyl
iPr	Isopropyl
Nle	Norleucine
NMM	N-methylmorpholine
Sar	Sarcosine
THF	Tetrahydrofuran

The first group of compounds of this invention are of the formula:

$$
\begin{array}{c}
O = CR^1 \\
| \\
(CH_2)_n \\
\overset{X}{\underset{\parallel}{}} \qquad \overset{O}{\underset{\parallel}{}} \qquad | \\
H_2N - C - NH - Y - C - (Z -) NH - CH - \underset{\underset{O}{\parallel}}{C} - R^2 \qquad \text{(I)}
\end{array}
$$

wherein

X   is selected from NR, S and O;

Y   is an unsubstituted or substituted, linear or branched linking moiety selected from saturated and unsaturated hydrocarbon groups containing from 1 to about 15 linking atoms, and optionally containing one or more heteroatoms and/or one or more cyclic structures;

Z   is optional and, where present, is selected from Gly (most preferably), and from Ala, Sar and βAla;

R^1 and R^2 are independently substituents selected from -OR (including hydroxyl), -NR$_2$ (including -NH$_2$ and -NHR),

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-NHNH$_2$ and -SR, and where one of R^1 and R^2 may additionally be selected from

$$\text{Ser-R}^3, \quad \text{Thr-R}^3, \quad \text{Val-R}^3, \quad \text{Ala-R}^3, \quad \text{Leu-R}^3, \quad \text{Ile-R}^3,$$
$$\underset{\text{(OR}^4)}{|} \qquad \underset{\text{(OR}^4)}{|}$$

$$\text{Nle-R}^3, \quad \underset{\text{O}}{\overset{\displaystyle (CH_2)_m\text{-OR}^4}{NH-CH-\underset{\|}{C}R^3}}, \quad NH-(CH_2)_m-OR^4$$

and

$$NH-(CH_2)_m-\overset{\displaystyle O}{\underset{\|}{C}}-R^3$$

wherein R^3 is a substituent selected from -OR (including hydroxyl), -NR$_2$ (including -NH$_2$ and -NHR), -NHNH$_2$ and -SR, and

R^4 is a substituent selected from groups of the form -R and acyl groups -COR;

5

n and (where present) m are independently 1, 2 or 3;

and wherein each R is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and substituted C$_1$-C$_8$ lower alkyls, C$_2$-C$_8$ alkenyls, C$_2$-C$_8$

10

alkynyls, C$_6$-C$_{14}$ aryls, C$_7$-C$_{14}$ alkaryls, C$_7$-C$_{14}$ aralkyls and C$_3$-C$_{14}$ cycloalkyls, from heteroatomic groups and, in the case of -NR$_2$, from cyclized groups forming (in attachment with the nitrogen atom) a 5-8

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membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

The compounds of the invention further include pharmaceutically acceptable base- or acid-addition salts of the compositions of Formula I. The pharmaceutical

5   compositions of the invention include such compounds (including salts thereof) formulated with a pharmaceutically acceptable excipient.

It will be seen that the X substituent of the form RN=, as well as the substituents R^1, R^2 and (where present) R^3 and R^4, are in turn each independently defined in terms of a further substituent R. With respect to these latter substituents

10   designated as R, hydrogen, as well as unsubstituted and substituted lower alkyl and single-ring aryl (including single-ring aralkyl and alkaryl) hydrocarbon moieties, are most generally preferred. Methyl, ethyl, isopropyl, tert-butyl and benzyl are examples of such preferred hydrocarbon substituents, with benzyl ($-CH_2(C_6H_5)$) being a particularly preferred aralkyl substituent. Homoatomic polycyclic structures

15   such as naphthalene, decalin, anthracene or phenanthrene may also be employed, although single-ring structures are presently preferred for cyclic substituents. Each R group in the compound may be independently selected, and need not be the same in each position in the compound.

Where R in such cases is chosen to be a heterocyclic group, the cyclic structure

20   may contain one or more heteroatoms selected from N, O and S, and may be mono- or polycyclic. The cyclic structure can be saturated, as in morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, imidazolidinyl, and other structures, or unsaturated or aromatic, as in imidazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyrazolyl, pyrrolyl, pyrrolinyl, pyridazinyl, pyrrodiazolyl,

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isothiazolyl, thienyl, thiazinyl, isoxazolyl, furazanyl and other structures. Polycyclic structures such as indolyl, quinolyl, quinazolinyl, phenoxazinyl, phenazinyl, phenothiazinyl, benzo[b]thienyl, phenanthrolinyl or others may be employed. Attachment of such cyclic R groups with the remainder of the compound may occur through a carbon or (provided that a point of bonding is present on a heteroatom) through a heteroatom within the heterocyclic group, or attachment may be achieved through, for example, an intermediate alkylene moiety which links the cyclic group with the remainder of the compound. Such cyclic R groups may also be substituted with pharmaceutically suitable substituents as is now discussed.

Where one or more of the R groups in the compound is itself additionally substituted, preferred substituents include hydroxyl, amino, lower (C_1-C_6) alkoxyl and alkyl, and, in the case particularly of aromatic R groups, the foregoing substituents as well as nitro and halogen (especially chloro and bromo) moieties. Such substituents on R may be used, for example, to alter bioactivity, solubility and/or biodistribution characteristics of the subject compounds. Where R includes an aryl group, substituents occurring on the *meta* and/or *para* positions (i.e., 3'- and/or 4'-positions) are most preferred. Preferred alkaryl forms of R thus include (3'-methyl)phenyl and (4'-methyl)phenyl groups. Multiply-substituted aryls, particularly where the substituent is small as in methyl or halo, are also useful. These preferred substituents on the R groups are also preferred for structures of the invention described hereinafter.

With regard particularly to the X-position, the most preferred substituent is of the form RN= (i.e., wherein the heteroatom nitrogen is double-bonded to the adjacent carbon). The preferred R substituents in this position are as discussed above, with

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hydrogen being most highly preferred (so as to yield an unsubstituted guanidinyl moiety). Lower alkyl and single-ring aryl (including single-ring aralkyl and alkaryl) moieties are also preferred. Oxygen- and sulfur-containing analogs of guanidinyl moieties, wherein X= is O= or S= (hereinafter referred to "guanidinyl analogs," specifically of a urea or thiourea form), may also be employed.

With regard particularly to the substituents R^1, R^2, R^3 and R^4, those of the form -OR, with choices of R therein yielding hydroxyl substituents (i.e., R is hydrogen), as well as those yielding unsubstituted and substituted lower alkoxy and single-ring aryloxy, and unsubstituted and singly-substituted lower alkylamino substituents, are preferred. As noted above, R may be the same or different in each position in R^1, R^2, R^3 and R^4. Where such a substituent yields a structure of the form -OR other than hydroxyl (i.e., R is other than hydrogen), structures of the form $-OCH_3$, $-OCH_2(C_6H_5)$ and $-O(C_6H_5)$ (i.e., -R is $-CH_3$, $-CH_2(C_6H_5)$ or $-(C_6H_5)$ are especially preferred and are believed to protect the resultant compound from metabolism in the *in vivo* environment, and may also tend to enhance the ability of the compound to interact with receptor sites on cells or other structures in the body, thereby increasing the activity of the compound. Likewise, where R^1, R^2 and/or R^3 are of the form $-NR_2$, amidating substituents such as those of the form $-NH_2$, $-NHCH_3$ and $-NHCH_2CH_3$ (i.e., each -R is hydrogen, or one -R is hydrogen and the other is $-CH_3$ or $-CH_2CH_3$), and amidating substituents wherein the nitrogen is itself substituted with one or two aromatic groups (i.e., one or two R groups in $-NR_2$ is of an aryl, alkaryl or aralkyl form as defined above) are preferred.

With respect to R^1, R^2, and R^3, bulky esterifying groups, such as aromatic esterifying groups, of the form -OR are particularly preferred, and the benzyloxy $(-OCH_2(C_6H_5))$ group is particularly preferred for each of R^1, R^2 and R^3. Similarly,

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with respect to R^4, it is especially preferred that R therein be an aromatic group, particularly benzyl or benzoyl. As described above, substituents on such aromatic groups including hydroxyl, amino, lower alkoxyl and alkyl substituents, and especially electron-withdrawing substituents such as halogen and nitro groups, are
5 useful in one or more positions on the aromatic structure. Examples of preferred substituents (aromatic and otherwise) in these positions include those wherein R is p-chlorobenzyl, benzyloxymethyl, trityl (triphenylmethyl), t-butyl, cinnamyl, other substituted benzyl or substituted methyl, alkyl, cycloalkyl, phenyldimethylsilyl, t-butyldimethylsilyl and triisopropylsilyl.

10 Where an amidating group of the structure $-NR_2$ is to be cyclic in form, the N-morpholinyl heterocyclic structure is preferred. Where one or more R groups in such a structure includes an aromatic group, each may be further substituted in one or more positions as described above. Unsubstituted or substituted benzyl substituents are particularly preferred in such structures.

15 The R^2 substituent, or alternatively the R^1 substituent, may comprise, in addition to a portion containing a group of the form R as described above, an amino acid structure chosen from various amino acid residues as well as analogs thereof. As an example of one such form of R^2 (or R^1), the structure -Val-R^3 set forth above defines a carboxyl-substituted form of the amino acid valine, wherein the
20 carboxyl-substituent is R^3 as defined above. Where R^3 is chosen to be hydroxyl (as in -Val-OH), the resulting R^2 group ig the amino acid valine. Similarly, R^3 may be chosen to be an esterifying or amidatinq group (as in, e.g., -Val-OR, -Val-NH_2 or -ValNHR$_2$), wherein R is chosen as described above and is preferably a lower alkyl or single-ring aryl (e.g., benzyl or phenyl) group. Likewise, R^2 (or R^1) groups
25 of the form -Ala-R^3, -Leu-R^3, -IleR^3, -Nle-R^3 and -Thr-R^3 refer to the carboxyl-

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substituted amino acids alanine, leucine, isoleucine, norleucine, and threonine, wherein R^3 may be hydroxyl or other substituent as described above.

The groups

——Ser-R^3 and   ——Thr-R^3
       | |
     (OR⁴)   (OR⁴)
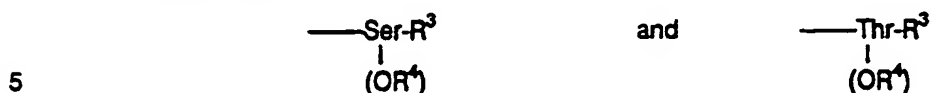
as used above with respect to R^2 (or R^1) define carboxyl-substituted serine and threonine moieties having, additionally, a substituent R^4 on the oxygen of the amino acid side chain. Serine-derived moieties are particularly preferred in this position. Where R^4 in the above formula is chosen to be hydrogen, and R^3 is hydroxyl, a simple serine or threonine moiety results.

The other forms recited in Formula I above for R^2 (or R^1) represent analogs of serine wherein the side chain and/or the carboxylate group of the amino acid has been modified or truncated. Serine is especially preferred as the R^2 substituent, particularly when substituted at R^3 and/or R^4 (most preferably in both positions) with a bulky substituent for R therein. As noted above, it is especially preferred in such structures that the substituent R^4 on the side chain position be selected from bulky substituents including, for example, benzyl, p-chlorobenzyl, benzyloxymethyl, trityl (triphenylmethyl), t-butyl, cinnamyl, other substituted benzyl, substituted methyl, alkyl, cycloalkyl, phenyldimethylsilyl, t-butyldimethylsilyl and triisopropylsilyl groups, with the benzyl group (forming a side chain ether functionality) being especially preferred. Acyl substituents, including lower acyl substituents such as acetyl groups, are also useful.
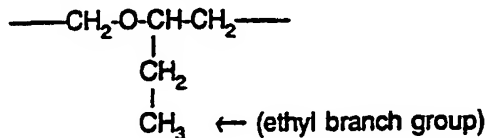
-20-

As will be discussed in more detail below, the linking moiety Y is preferably selected in conjunction with the other portions of the claimed compounds (most particularly the optional group Z, and the methylene moieties enumerated by the integer n) in a manner which provides suitable spacing between the guanidinyl (or guanidinyl-analog) group and the carbonyl-containing group designated as -COR^1. When the linking moiety Y is of a hydrocarbon chain form, the preferred length of the linking portion of the chain is from about 2 to about 5 methylene or other atomic residues (noninclusive of carbons or other groups appended as branches or substituents to the linking portion of the alkyl chain). Simple alkylene chains of the form -(CH_2)-$_r$, wherein r is an integer of from 1 to about 8, and preferably 1 to about 5 where the optional Z group is not present, are preferred for the linking group Y. Such chains may be used to yield des-α-amino forms of arginine and homologs thereof in the structures of the present compounds.

As used herein, the "linking portion" of Y refers to that portion of Y which effectuates the intramolecular spacing between the two residues of the compound adjacent to the Y group, i.e., the guanidinyl (or guanidinyl-analog) residue bonded to the left of Y and the

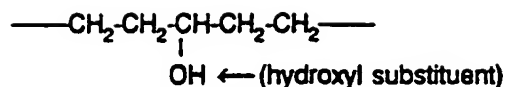$$\overset{O}{\overset{\|}{-\!\!-\!\!C}} - (Z-) \cdots$$
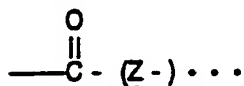
residue bonded to the right of Y. Thus, portions of Y which do not contribute to this inter-residue spacing within the subject compounds, such as branch groups or substituents as in, for example,

$$-CH_2\text{-}O\text{-}CH\text{-}CH_2-$$
$$\overset{|}{CH_2}$$
$$\overset{|}{CH_3} \leftarrow \text{(ethyl branch group)}$$

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or

$$\text{---CH}_2\text{-CH}_2\text{-CH-CH}_2\text{-CH}_2\text{---}$$
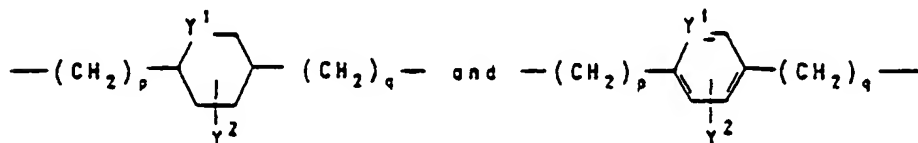$$\underset{\text{OH} \ \leftarrow \text{(hydroxyl substituent)}}{|}$$

are not to be considered a part of the linking portion of Y. Accordingly, in the examples of Y depicted above, the linking portions affording spacing between the adjacent residues in the compound comprise, respectively, 4 linking atoms (-C-O-C-C-) and 5 linking atoms (-C-C-C-C-C-). As noted, the number of such linking atoms, and the resultant spacing distance provided by the linking moiety Y, may be modified in conjunction with other portions of the compound so as to provide desired intramolecular spacing as discussed hereinafter.

Unsaturated linking moieties Y containing double and/or triple bonds will also preferably include from about 2 to about 5 carbon atoms in the linking portion of the chain, and may include from about 1 to about 3 double or triple bonds. As seen above, one or more heteroatoms such as O, S or N may be included as substituents within the structure of the linking moiety, as for example to form an ether, thioether or secondary or tertiary amino linking structure. However, the compounds of the invention do not include those wherein Y is substituted with a primary amino group so as to form (in conjunction with the adjacent portions of the compound) an arginine (Arg) residue.

Where the linking moiety Y includes a cycloalkyl or aryl portion within its structure, such a moiety may be of the form

$$-(CH_2)_p - \underset{\underset{Y^2}{|}}{\overset{\overset{Y^1}{|}}{\bigcirc}} - (CH_2)_q - \quad \text{and} \quad -(CH_2)_p - \underset{\underset{Y^2}{|}}{\overset{\overset{Y^1}{|}}{\bigcirc}} - (CH_2)_q - $$

-22-

wherein Y^1 is carbon or an O, S or N heteroatom, and Y^2 is an optional substituent of the form discussed above with respect to substituents on R, especially hydroxyl, amino or lower (C_1 to C_8) alkoxyl or alkyl, or other pharmaceutically suitable substituents such as nitro or halogen (especially chloro or bromo) groups. The integers p and q range independently from 0 to about 5, preferably 0 to 3, and most preferably the sum of p and q is 2. As with the linear linking groups Y described above, the choice of cyclic structures for Y (with or without appended (poly)methylene moieties) may be made in conjunction with other structures in the compound so as to achieve a desired intramolecular spacing between the guanidinyl residue and the remainder of the molecule. In this regard, the cyclic portion of each Y group depicted above contributes spacing equivalent to about 3-4 straight-chain methylene units; other cyclic or alternative linking structures may likewise be evaluated to ascertain their contribution to intramolecular spacing.

The optional group Z, where present, provides a linking function in addition to that of group Y and, therefore, may also be chosen in conjunction with the other structures in the compound to afford a desired intramolecular spacing. It is most preferred that Z be absent, particularly where the linking moiety Y contains about 5 or more linking atoms. Where present, a preferred Z group is the glycine residue (Gly), i.e.,

$$
\overset{\text{O}}{\overset{\|}{\text{——NH-CH}_2\text{-C——}}}
$$

which provides a linking portion comprising 3 linking atoms (-N-C-C-). Alanine (Ala), sarcosine (Sar) and β-alanine (β-Ala) are also preferred in this position, and these provide 3 (Ala and Sar) or 4 (β-Ala) linking atoms in their structures. Each of these examples provides, as is preferred, an amino group at the left-hand terminus of Z and a carbonyl group at the right-hand terminus, thereby allowing

-23-

peptide-like amide bonding to each adjacent portion of the compound. Where Z is absent in the compound, it is desirable to increase the lengths of the linking portions of the Y group and/or the methylene moieties enumerated by n so as to maintain the desired intramolecular spacing.

5

The portion of the present compounds having the structure

$$
\begin{array}{c}
O = CR^1 \\
| \\
(CH_2)_n \\
| \\
-NH - CH - C- \\
\| \\
O
\end{array}
$$
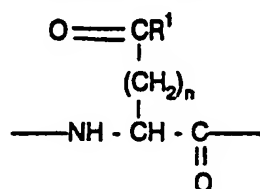
10

corresponds to the aspartic acid (Asp) amino acid residue and homologs and derivatives thereof. Where n is chosen to be 1 and R^1 is hydroxyl, a simple Asp regidue results. (Of course, at physiological pH, the carboxylic acid hydrogen in such a residue, and in homologs thereof, will be substantially ionized to the carboxylate form.)

15

It is particularly preferred that R^1 in the above structure be of the form -OR, where R is selected from hydrogen or, more preferably, from bulky substitutents including benzyl, phenyl, and others described above with respect to R^1, R^2, R^3 and R^4.

It has been discovered in investigations relating to the invention that significant inhibition of cell adhesion may be achieved in the present compounds by providing, in a nonpeptide structure as is here described, a carbonyl-containing functional group (preferably a carboxyl or ester functional group) in appropriate proximity to a guanidinyl or guanidyl analog functional group. It is believed to be useful in this regard to maintain the intramolecular distance between such

20

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carbonyl-containing and guanidino groups within a fairly broad range that may be adjusted by using suitable choices of linking moieties Y and Z. Thus, with respect to the Asp-analog residues depicted in the formula immediately above, such proximity may be modified by selecting varying numbers of methylene moieties as enumerated by the integer n. Thus, where n is 1 (as in a simple Asp residue), the number of linking atoms in the residue separating the carbonyl functional group from the remainder of the compound (in the direction of the guanidinyl functional group) is, effectively, 3. These linking atoms are of the structure

$$[O \!=\!\!= CR^1]$$
$$|$$
$$C$$
$$|$$
$$\text{---} N\text{-}C \cdots$$
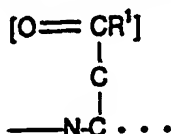
(where the carbonyl-containing functional group on the "side chain" on the residue is depicted in brackets).

Similarly, with reference to Formula I, it is seen that the guanidinyl (or guanidinyl-analog) functional group at the left-most position in the molecule is separated from the R^1-attached carbonyl group by a linking structure that may be depicted as

$$[\text{guanidinyl-analog}]\text{-}Y\text{-}C\text{-}(Z\text{-})N\text{-}C\text{-}(C)_n\text{---}[\text{carbonyl-}R^1]$$
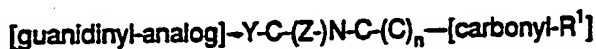
The total number of linking atoms in the linking portion of this structure (L^{tot}) is equal to $L^Y + L^Z + n + 3$, where L^Y and L^Z are the number of linking atoms (or their equivalent) in Y and Z, respectively, and n is an integer as defined above. It is generally preferred that L^{tot} be in the range of from about 7 to about 20, and most preferably from about 9 to about 12. Those practicing the invention will, in view of the present disclosure, be able to choose suitable linking groups so as to attain a desired intramolecular spacing for the product compounds.

As noted above, particularly preferred compounds include those within Y and/or
Z represent simple alkylene linking groups, X is HN= and one or more of R^1, R^2
and (if present) R^3 and R^4 are esterifying substituents. Among such preferred
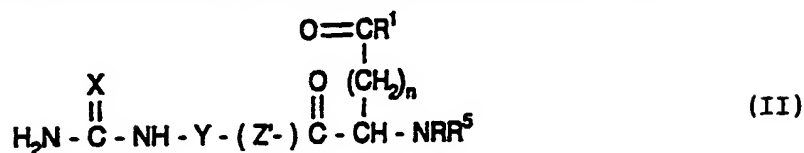compounds are

and
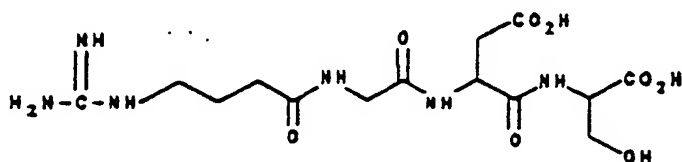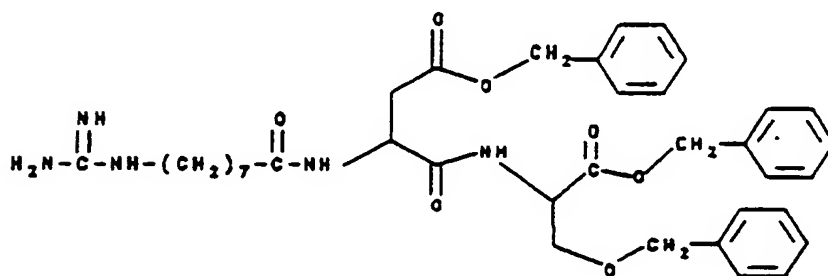
wherein L^{tot} is, respectively, 10 ($L^Y = 3$, $L^Z = 3$) and 11 ($L^Y = 7$, Z absent). The
latter compound is especially preferred. Also preferred are compounds of the form
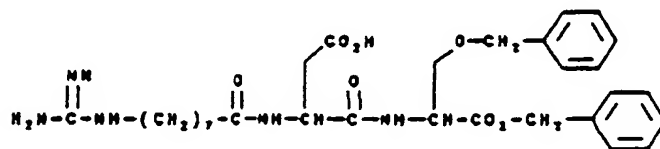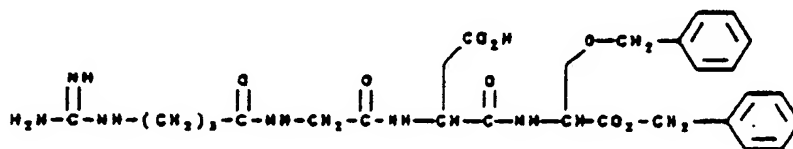
-26-

A second group of compounds of the invention has the structure

$$\begin{array}{c} O{=}CR^1 \\ | \\ X \qquad\qquad O\ (CH_2)_n \\ \| \qquad\qquad \| \ | \\ H_2N - C - NH - Y - (\ Z'-)\ C - CH - NRR^5 \end{array} \qquad (II)$$
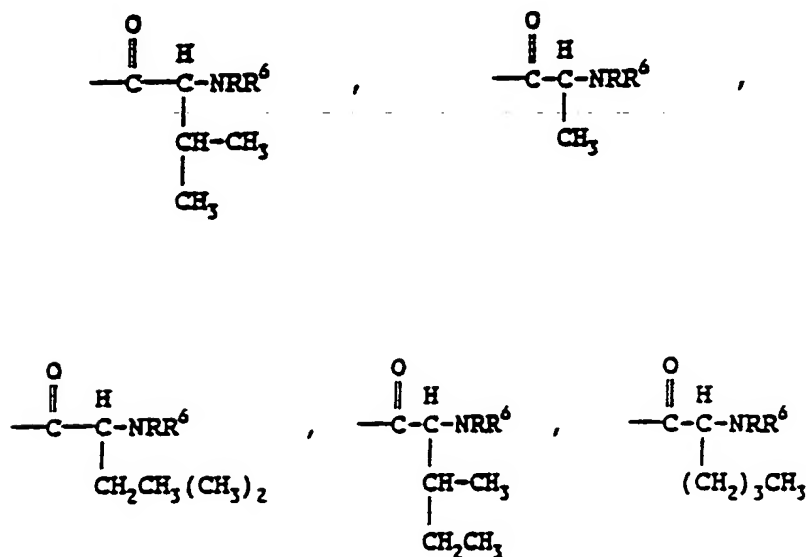
5   wherein X is selected from NR, S and O;

Y   is an unsubstituted or substituted, linear or branched linking moiety selected from saturated and unsaturated hydrocarbon groups containing from 1 to about 15 linking atoms, and optionally containing one or more heteroatoms and/or one

10   or more cyclic structures;

R^1 is a substituent selected from -OR (including hydroxyl), -NR_2 (including -NH2 and -NHR), -$NHNH_2$ and -SR;

R^2 is is selected from -R, acyl groups -COR, alkyloxycarbonyl groups of the form

15
$$\begin{array}{c} -\!\!-\!\!C\text{-}OR, \\ \| \\ O \end{array}$$

and from groups of the form

$$\begin{array}{cc}
\begin{array}{c}
O \\
\| \quad H \\
-\!\!-C-\!\!-C\text{-}NRR^6 \\
| \\
CH\text{-}CH_3 \\
| \\
CH_3
\end{array}
\quad , &
\begin{array}{c}
O \\
\| \quad H \\
-\!\!-C-C\text{-}NRR^6 \\
| \\
CH_3
\end{array}
\quad ,
\end{array}$$

$$\begin{array}{ccc}
\begin{array}{c}
O \\
\| \quad H \\
-\!\!-C-\!\!-C\text{-}NRR^6 \\
| \\
CH_2CH_3(CH_3)_2
\end{array}
\quad , &
\begin{array}{c}
O \\
\| \quad H \\
-\!\!-C-C\text{-}NRR^6 \\
| \\
CH\text{-}CH_3 \\
| \\
CH_2CH_3
\end{array}
\quad , &
\begin{array}{c}
O \\
\| \quad H \\
-\!\!-C-C\text{-}NRR^6 \\
| \\
(CH_2)_3CH_3
\end{array}
\end{array}$$

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$$-\overset{\overset{\text{O}}{\|}}{\text{C}}-\overset{\overset{\text{H}}{|}}{\underset{\underset{\text{CH}_3}{|}}{\underset{\text{CH}-\text{OR}^6}{|}}}\text{C}-\text{NRR}^6 \quad , \quad -\overset{\overset{\text{O}}{\|}}{\text{C}}-\overset{\overset{(\text{CH}_2)_a-\text{OR}^6}{|}}{\text{CH}}-\text{NRR}^6 \quad , \quad \text{and}$$

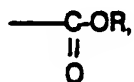$$-\overset{\overset{\text{O}}{\|}}{\text{C}}-(\text{CH}_2)_a - \text{OR}^6$$
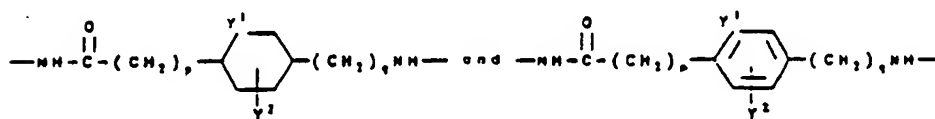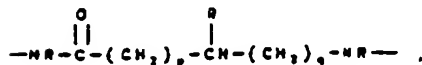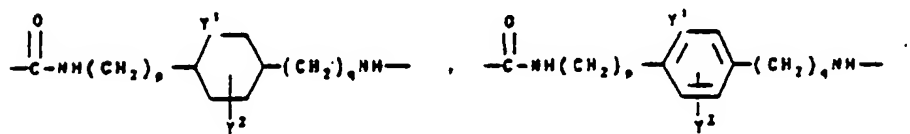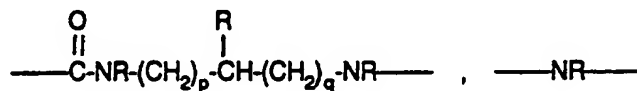
wherein R^4 and R^6 are substituents independently selected from groups of the form -R and acyl groups -COR, and R^6 may additionally be selected from alkoxycarbonyl groups of the form

$$-\overset{}{\underset{\overset{\|}{\text{O}}}{\text{C}}}-\text{OR},$$

5

Z' is a suitable linking moiety, preferably selected from those of the form

$$-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{NR}-(\text{CH}_2)_p-\overset{\overset{\text{R}}{|}}{\text{CH}}-(\text{CH}_2)_q-\text{NR}- \quad , \quad -\text{NR}-$$

$$-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{NH}(\text{CH}_2)_p-\underset{\underset{\text{Y}^2}{|}}{\overset{\overset{\text{Y}^1}{|}}{\bigcirc}}-(\text{CH}_2)_q\text{NH}- \quad , \quad -\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{NH}(\text{CH}_2)_p-\underset{\underset{\text{Y}^2}{|}}{\overset{\overset{\text{Y}^1}{|}}{\bigcirc}}-(\text{CH}_2)_q\text{NH}- \quad ,$$

$$-\text{NR}-\overset{\overset{\text{O}}{\|}}{\text{C}}-(\text{CH}_2)_p-\overset{\overset{\text{R}}{|}}{\text{CH}}-(\text{CH}_2)_q-\text{NR}- \quad ,$$

$$-\text{NH}-\overset{\overset{\text{O}}{\|}}{\text{C}}-(\text{CH}_2)_p-\underset{\underset{\text{Y}^2}{|}}{\overset{\overset{\text{Y}^1}{|}}{\bigcirc}}-(\text{CH}_2)_q\text{NH}- \quad \text{and} \quad -\text{NH}-\overset{\overset{\text{O}}{\|}}{\text{C}}-(\text{CH}_2)_p-\underset{\underset{\text{Y}^2}{|}}{\overset{\overset{\text{Y}^1}{|}}{\bigcirc}}-(\text{CH}_2)_q\text{NH}-$$

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wherein

Y^1 comprises carbon or an O, S or N heteroatom;

Y^2 is an optional substituent selected from hydroxyl, amino, lower (C_1-C_8) alkoxyl and alkyl, nitro and halogen moieties;

5   p and q are independently integers of from 0 to about 5;

n and (where present) m are independently 1, 2 or 3; and wherein each R is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and subtstituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls,

10   C_7-C_{14} alkaryls, C_7-C_{14} aralkyls and C_3-C_{14} cycloalkyls, from heteroatomic groups and, in the case of $-NR_2$, from cyclized groups forming (in attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

15   As with the compounds of Formula I, the compounds of the invention further include pharmaceutically acceptable base or acid-addition salts of the compounds of Formula II. The pharmaceutical compositions of the invention include such compounds and salts thereof formulated with a pharmaceutically acceptable excipient.

20   It will be seen that the compounds of Formula II are structurally similar to those of Formula I except insofar as the peptide bond orientation of the groups to the right of Z' has been reversed. Accordingly, Z' is most conveniently selected to accommodate this reverse orientation by providing an amino group suitable for bonding to the carbonyl group of the succeeding reverse-orientation Asp-analog.

25   Likewise, the substituents for R^5 correspond (except insofar as orientation) to those

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given above for R^2 in Formula I, and also allow for suitable substitution (in the form of R^6) at the amino terminus appearing in R^5.

It is preferred in Formula II that D-enantiomeric residues be utilized in the reversed-orientation residues, i.e., in the Asp residue (to the right of Z') and, if present, in any optically active site in R^5. The other preferred substituents and preferred structures described above for Formula I are likewise preferred for the compounds of Formula II.

A particularly preferred R^5 substituent is that of the substituted or unsubstituted reverse form of serine, i.e.,

$$\underset{\overset{\text{O}}{\underset{\text{C-CH-NRR}^6}{\|}}}{\overset{\text{CH}_2\text{-OR}^4}{\vert}}$$
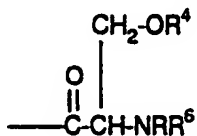
preferably in the D-enantiomeric form. As with the analogous R^2 substituent discussed above with respect to Formula I, bulky substituents (as described above) are preferred for R^4, and such bulky substituents are also preferred in the R^8 position.

The linking group Z' is preferably a lower diaminoalkane structure, e.g., of the form $-NH(CH_2)_m-NH-$, where m is (as defined) 1, 2 or 3. Likewise, Y is preferably a 1 to 8 carbon methylene chain as discussed above for Formula I.

As with the analogous portions of Formula I, the Y and Z' moieties in Formula II are conveniently selected so as to maintain a desired intramolecular spacing in the compound between the guanidinyl (or guanidinyl-analog) group and the carbonyl-containing group designated as $-COR^1$. In this case, the total number of

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linking atoms in the linking portion of Formula II (L^{tot}) is equal to $L^Y + L^Z + n + 2$, where L^Y and L^Z are the number of linking atoms (or their equivalent) in Y and Z, respectively, and n is the integer as defined above. In Formula II, as with Formula I, it is generally preferred that L^{tot} be in the range of from about 7 to about 20, and most preferably from about 9 to about 12.

Particularly preferred structures within the scope of Formula II include:
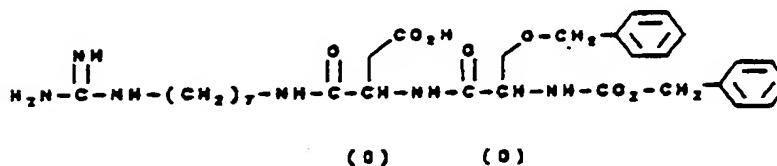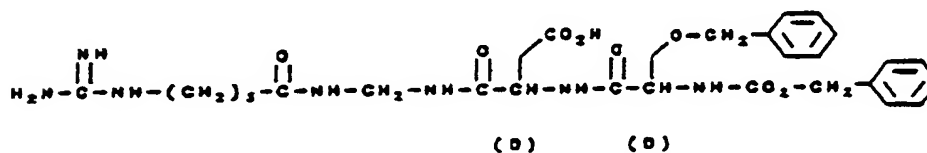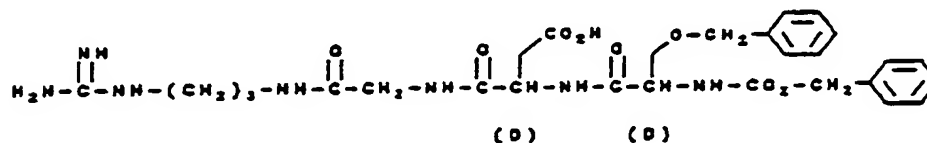
The following examples are given to illustrate methods whereby the present compounds may be synthesized, formulated and tested, and are not intended to limit the scope of the present invention.

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EXAMPLE 1

Synthesis of 8-guanidinyloctanoyl-(O-phenylmethyl)-aspartyl-(O-phenylmethyl) serine phenylmethyl ester

The title compound was prepared by first dissolving 1.02 g (1.72 mmole) of the starting ester
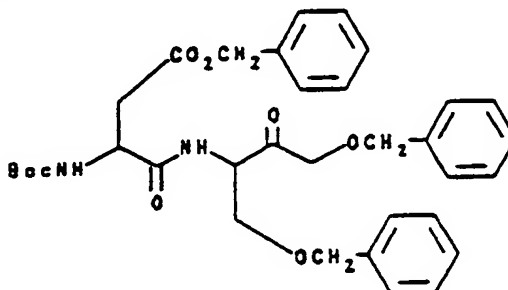
in 4 ml of 4M HCl in ethyl acetate and allowing the solution to stand for 2 hours at room temperature. After 2 hours the volatiles were removed at reduced pressure and the residue dried with toluene. The residue was dissolved in 8 ml of pyridine, and then 0.38 g (1.84 mmole) of dicyclohexylcarbodiimide (DCC) and 0.52 g (2.0 mmole) of the t-butyloxycarbonyl (BOC) protected acid

$$BOC\text{-}NH\text{-}(CH_2)_7\text{-}CO_2H$$

was added followed by addition of 0.21 g (2.0 mmole) of N-methyl morpholine. The next day the reaction mixture was filtered and the residue evaporated at reduced pressure. The residue as taken up in ethyl acetate, washed with 5% citric acid, with 5% NaHCO$_3$, dried and evaporated to give 1.16 g of red oil. Chromatography on a chromatatron with 80% ethyl acetate gave 0.72 g of white foam (55.4% yield). Crystallization from petroleum ether/ethyl acetate gave an analytical sample of the intermediate t-butyloxycarbonyl-8-aminooctanoyl-(O-phenylmethyl)-aspartyl- (O-phenylmethyl) serine phenylmethyl ester. Mp: 65-73;

-32-

IR (CHCl₃): 3342 (NH), 1740 (ester), 1686 (amide I), 1644 (urethane); ^{1}H-NMR (CDCl₃): 7.18-7.40 (m, 16H, aromatic + NH), 4.86-4.94 (m, 1H, NCHCO), 4.68-4.74 (m, 1H, NCHCO), 2.16 (t, J=7.5, 2H, CH₂CON), 1.44 (5, 9H, t-Ba); $[\alpha]^D$=-15.4°. Anal. calcd. for $C_{43}H_{53}N_3O_9H_2O$: C, 66.75; H, 7.16; N, 5.43; found: C, 66.96; H, 7.28; N, 5.83.

A 0.15 g (.020 mmole) portion of the intermediate ester was dissolved in 5ml of 4M HCl in ethyl acetate. After two hours the volatiles were removed at reduced pressure. The residue was suspended in 2 ml of water, 59.3 mg (0.43 mmole) of potassium carbonate, and 35.4 mg (0.28 mmole) of aminoimino sulfonic acid was added. An immediate white precipitate formed. The next day the reaction mixture was filtered to give 0.21 g of the title compound in the form of a gummy yellow golid. The structure of the product (1) was
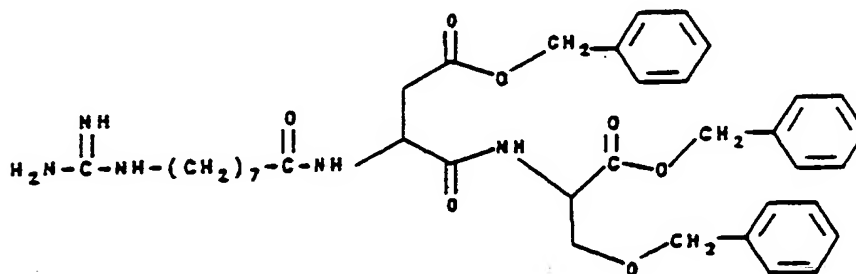

An alternative synthesis of the title compound was performed as follows. A 0.59 g (1.0 mmole) portion of the starting ester depicted above was dissolved in 5 ml of 4M HCl in ethyl acetate and allowed to stand overnight. The next day the volatiles were removed at reduced pressure and the residue dried with toluene.

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An 0.21 g (1.1 mmole) portion of water soluble carbodiimide was added to the residue. A solution of 0.20 g (0.99 mmole) of the guanidinylated compound

$$\underset{\underset{H_2N-C-NH-(CH_2)_7-CO_2H}{\overset{NH}{\|}}}{}$$
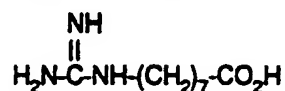
5   and 0.22 g (2.17 mmole) of N-methylmorpholine (NMM) in 6 ml of pyridine was then added to the residue. The next day the pyridine was removed at reduced pressure and the residue shaken with 25 ml of water. Filtration gave 0.34 g af the title compound in the form of a gummy solid which was chromatographed on a 2-inch long C-4 reverse phase column. Elution with 45 : 55 : 0.5 acetonitrile :

10   water : acetic acid gave 0.16 g (23% yield) of a white gum with a k^1=0.66. ^{1}H-NMR (DMSO-d_6): 7.2-7.34 (m, 10H, aromatic), 4.95-5.10 (m, 4H, CH$_2$(C$_6$H$_5$), 4.64-4.70 (m, 1H, NCHCO), 4.47-4.52 (m, 1H, NCHCO), 3.75 (d of d, J=9.9 & 4.1), 3.58 (d of d, J=9.9 & 3.5), 2.97 (t, J=6.8, 2H, CH$_2$-guanidine), 2.02 (t, J 7.2, 2H, CH$_2$CO).)

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EXAMPLE 2

General procedure for peptide condensation

As a general procedure for synthesis of the present compounds, condensations may be performed in DMF with DCC as the condensing agent. The amino acid DCHA salts are neutralized by suspending them in 1M aqueous NaHSO$_4$ and repeatedly extracting with ethyl acetate. The combined extracts are dried over Na$_2$SO$_4$, filtered, and evaporated. The BOC protecting groups are removed when needed by dissolving the amine in ethyl acetate and adding three equivalents of HCl in the same solvent. When the cleavage is complete, the volatiles are removed at reduced pressure and the residue dried by adding toluene and removing it aqain on the rotary evaporator. The hydrochloride salt is neutralized in situ by adding NMM to the DMF reaction mixture. After the condensation is complete the dicyclohexylurea is removed by filtration and the volatiles removed at reduced pressure. The peptide residue is taken up in ethyl acetate, washed with aqueous citric acid, washed with aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and evaporated at reduced pressure.

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EXAMPLE 3

Synthesis of
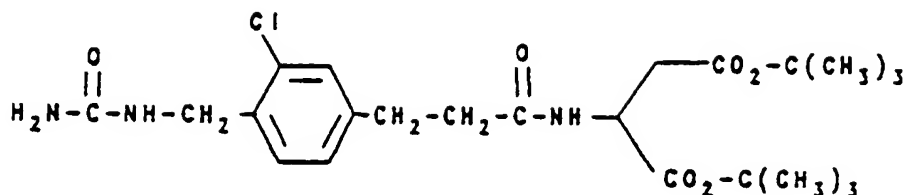
(2)

The depicted compound, 4-ureidomethyl-3-chloro-phenylpropionyl aspartic acid-di-t-butyl ester (2), is prepared via the intermediate products now described.

4-Chloromethyl-3-chlorophenylpropionic acid methyl ester. A mixture of 3-chlorophenylpropionic acid methyl ester and three equivalents of methanol in concentrated aqueous HCl is stirred rapidly as HCl gas is bubbled through the mixture. After all the propionic acid has reacted, the reaction mixture is cooled and the crude benzyl chloride collected. Recrystallization gives the desired intermediate compound free of the 2-chloromethyl isomer.

4-Ureidomethyl-3-chlorophynelpropionic acid-methyl ester (2). A mixture of the above benzyl chloride and an equivalent of ferric chloride are stirred as cyanogen chloride is passed through the reaction mixture with careful exclusion of moisture. The resulting isocyanide dichlorideferric chloride complex is not isolated, but treated with anhydrous ammonia. An extractive work-up with chloroform gives the desired urea.
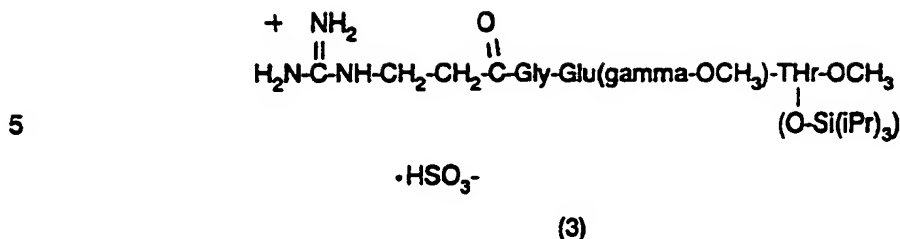
-36-

4-Ureidomethyl-3-chlorophenyl propionic acid. The methyl ester from above is saponified according to standard methods using one equivalent of lithium hydroxide in methanol.

4-Ureidomethyl-3-chlorophenylpropionyl aspartic acid-di-t-butyl ester (2). A solution of the above acid and one equivalent each of DCC and the hydrochloride salt of di-t-butyl aspartic acid (Bachem Biosciences) in DMF are stirred as one equivalent of NMM is added. The next morning, the dicyclohexyl urea is removed by filtration and the filtrate evaporated at reduced pressure. The residue is taken up with ethyl acetate, washed with sequential portions of aqueous citric acid and aqueous sodiun bicarbonate, dried, and evaporated to give the desired product.

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EXAMPLE 4

Synthesis of

$$+ \quad NH_2 \qquad O$$
$$\overset{\parallel}{H_2N-C-NH-CH_2-CH_2-C-Gly-Glu(gamma-OCH_3)-THr-OCH_3}$$
$$\overset{\mid}{(O-Si(iPr)_3)}$$

5

·HSO₃⁻

(3)

The depicted compound, β-guanidinylpropionyl-glycyl-glutamyl(gamma-methyl ester)-threonine (β-triisopropyl silyl ether) (3), is prepared according to the following

10   steps.

BOC-glutamyl(gamma-methyl ester)-threonine methyl ester. The BOC-protected dipeptide is prepared according to standard procedures by sequential solution-phase coupling of BOC-Glu(gamma-methyl ester) and Thr methyl ester.

BOC-glycyl-glutamyl(gamma-methyl ester)-threonine methyl ester. This BOC-

15   protected tripeptide is prepared from the foregoing deprotected compound by coupling BOC-Gly with DCC in DMF/NMM in the usual fashion.

FMOC-β-alanyl-glycyl-glutamyl(gamma-methyl ester)-threonine methyl ester. The BOC-protected tripeptide compound is deprotected according to the general procedure and coupled with FMOC-β-Ala in DMF/NMM using DCC activation to

20   yield the tetrapeptide.

β-Guanidinylpropionyl-glycyl-glutamyl(gamma-methylester)-threonine(β-triisopropyl silyl ether) (4). Treatment of the above compound with triisopropyl silyl chloride

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and imidazole in DMF gives the fully protected tetrapeptide. The protected tetrapeptide above is dissolved in dichloromethane and one equivalent of piperidine is added. When cleavage of the FMOC group is complete, the volatiles are removed at reduced pressure. The resulting free amine is immediately dissolved in acetonitrile and one equivalent of aminoiminomethane sulfonic acid is added. The desired guanidine compound is collected and dried.

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EXAMPLE 5

Synthesis of
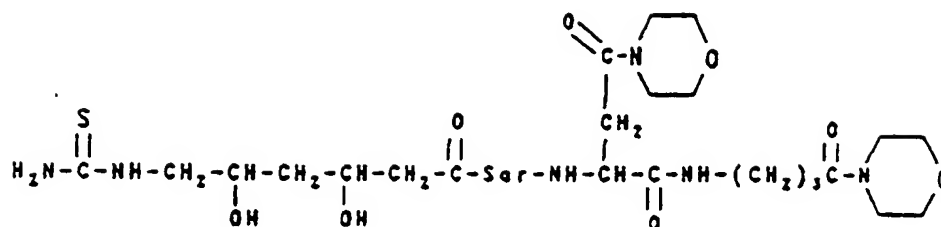
(4)

The title compound, 6-thioureido-4(S)-6(R)-dihydroxyhexanoyl-N-methylglycyl-
β-morpholinamide-aspartyl-4-aminobutanoyl morpholine amide (4) is prepared as
follows.

4-Aminobutanoic acid morpholine amide. BOC-gamma-aminobutyric acid (Bachem
Biosciences) is dissolved in dry THF along with one equivalent of
N-methylmorpholine. The solution is cooled to -15°C and one equivalent of
isobutylchloroformate is added dropwise. After 10 minutes at -15°C, one
equivalent of morpholine is added and the resulting slurry stirred overnight with
warming to room temperature. The next morning, the reaction mixture is diluted
with ethyl acetate, washed with aqueous citric acid, and washed with aqueous
sodium bicarbonate. The organic solution is dried with sodium sulfate, filtered, and
saturated with HCl gas. After one hour, the mixture is diluted with ether and the
desired amine salt collected.

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BOC-L-aspartic acid(β-morpholino carboxamide) dicyclohexyl amide salt. BOC-L-aspartic acid (α-benzyl ester) (Advanced ChemTech) is dissolved in THF with an equivalent of N-methylmorpholine. The solution was cooled to -15° and one equivalent of isobutylchloroformate is added. After 10 minutes at -15°, one

5   equivalent of morpholine is added. The reaction mixture is stirred as it warms to room temperature overnight. The reaction mixture is diluted with ethyl acetate, washed with aqueous citric acid, washed with aqueous sodium bicarbonate, dried, and filtered to give a clear solution. The organic solution is shaken with 50 psi of hydrogen and palladium on carbon until the α-benzyl ester is gone. The solution

10   is filtered through a Celite pad and one equivalent of dicyclohexylamine is added. Ether is added and the acid salt precipitate collected.

5,6-Epoxy-3(S)-hydroxylhexanoic acid methyl ester. The 3(R)-hydroxy-5-hexanoic acid ester which is prepared by baker's yeast reduction of the 3-oxo compound as described by Bennett in *Tetrahedron Letters*, 1988, 29, 4865 is dissolved in

15   methylene chloride and allowed to react with one equivalent of meta-chloroperbenzoic acid to give the desired epoxides, as a mixture of diastereomers.

6-Amino-3(R)-5-dihydroxyl hexnoic acid ethy ester 3-t- butydimethylsily ether. The epoxides from above are treated with t-butyldimethylsilyl chloride and imidazole in DMF under the usual conditions. The crude silyl ether is dissolved in ethanol and

20   treated with sodium azide and amonium chloride. After the epoxide has been consumed, the reaction mixture is shaken under 50 psi of hydrogen in the presence of platinum oxide. The crude amine solution is filtered, diluted with ethyl acetate, and washed with aqueous bicarbonate solution. Drying and filtration gave the desired amine as a mixture of diasteromers.

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2,2-Dimethyl-4(S)-benzyloxycarbonyl aminomethyl-1,3-dioxane-6(R)-acetic acid
ethyl ester. The crude amine from the previous step is converted to its
benzyloxycarbonyl derivative with benzyloxychloroformate and sodium hydroxide
in water at pH 10. Ethyl acetate extraction gives the crude benzyloxycarbonate
5   which is treated with 2-methoxypropene and a mixture of anhydrous HF and HCl.
When ketalization is complete, the homogeneous aqueous solution is washed with
aqueous bicarbonate solution, dried over sodium sulfate, and evaporated. The
diasteromeric ketals are separated by chromatography to afford the desired
compound.

10   2,2-Dimethyl-4(S)-thioureidomethyl-1, 3-dioxane-6(R)-acetic acid ethyl ester. The
Cbz-protecting group is removed from the ester from above in the standard way
with hydrogenation using Pd/C in ethyl acetate. The liberated amine is reacted
with silicon tetrathiocyanate as described by Nevile and McGee in Organic
Synthesis, 1973, 5, 802. The amine and 25 mole percent of silicon tetrathiocyanate
15   is heated at 75° in toluene until the amine is consumed. Hydrolysis with aqueous
2-propanol, filtration, and evaporation of the filtrate gives a crude thiourea which
is crystallized prior to the next step.

2,2-Dimethyl-4(S)-thioureidomethyl-1, 3-dioxane-6(R)-acetic acid. The ester from the
preceding step is saponified in aqueous ethanol with one equivalent of lithium
20   hydroxide in the cold overnight. Careful acidification to pH 5 with 1M HCl gives
the titled free acid.

6-Thioureido-4-(S)-6(R)-dihydroxyhexanoyl-N-methylglycyl-β-morpholinamideaspartyl-
4-aminobutanoyl morpoholine amide (4). The derivatized aspartic acid dicylohexyl
amide from above is condensed with the 4-aminobutanoic acid morpholine amide,

also above. After cleavage of the BOC group from the dipeptide, a second
condensation with BOC-sarcosine (BAchem Biosciences) is effected. Removal of
the BOC group from this tripeptide is followed by condensation with
2,2-dimethyl-4(S)-thioureidomethyl-1,3-dioxane-6(R)-acetic acid as prepared above.
The protected tetrapeptide is purified by column chromatography at this point. In
the final reaction, the ketal protecting group is removed with aqueous methanol
and HCl to afford the desired compound (4).

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EXAMPLE 6

Synthesis of

(5)
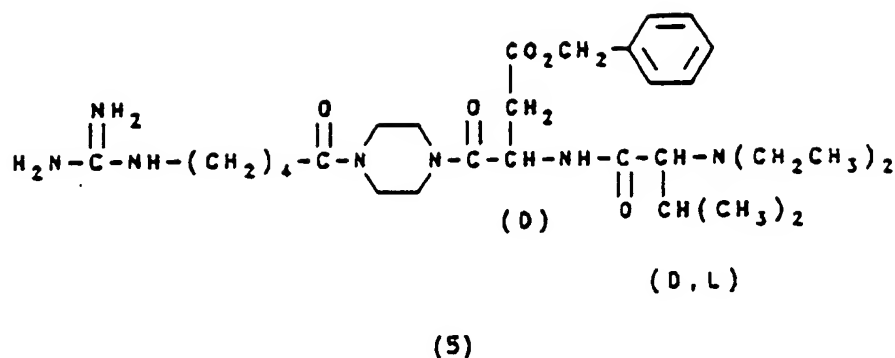
The title compound, 1-(5-guanidinylpentanoyl)-4-(N,N-diethyl-D,L-valyl-D-β-benzylaspartyl)-piperazine (5) is prepared as follows.

5   D,L-N,N-Diethylvaline. A mixture of the commercially available 3-methyl-2-oxo-butanoic acid sodium salt and an excess of diethylamine in water is stirred and cooled as one equivalent of sodium dithionite is added slowly. When the pyruvic acid has been consumed, the pH of the solution is adjusted with concentrated HCl to the isoelectric point of the product and the reaction mixture

10   diluted with an equal volume of alcohol. The amino acid is collected the next morning after storage in the refrigerator.

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FMOC-D-Aspartic acid (β-benzyl ester). A solution of D-aspartic acid (β-benzyl ester) (Bachem Biosciences) in aqueous sodium carbonate solution is cooled to 0°C and diluted with a one-half volume of dioxane. A solution of one equivalent of 9-fluorenylmethyl chloroformate in one volume of dioxane is added and the

5   mixture stirred overnight as it warms to room temperature. The reaction mixture is diluted with water and extracted with ether. The aqueous phase is acidified to pH 2 and repeatedly extracted with the ethyl acetate. The combined organic extracts are washed with water, dried over MgSO₄, and evaporated to give an oil which solidifies upon standing.

10   FMOC-D-Aspartyl-(β-benzyl ester)-α-1-(4-butyloxycarbonyl)-piperazinyl amide. The 1-BOC derivative of piperazine is prepared as described by Carpino in *J. Org. Chem.*, 1983, 48, 611 and condensed with the ester from the preceding step using the DCC general procedure. The resulting amide is purified by flash chromatography on silica gel.

15   5-Guanidinylpentanoic acid. A solution of 5-aminopentanoic acid and one equivalent of potassium carbonate in water is stirred as one equivalent of aminoimino methane sulfonic acid is added in portions over 15 minutes. The resulting slurry is stirred at room temperature for 24 hours. The precipitate is collected and dried to give the desired product.

20   1-(5-Guanidinylpentanoyl)-4-(N,N-diethyl-D,L-valyl-D-β-benzylaspartyl)-piperazine (5). The FMOC group is removed from the above piperazinyl amide intermediate with piperidine and the resulting amine dried *in vacuo*. Condensation by the usual DCC method with the racemic N,N-diethylvaline from above gives a dipeptide as a mixture of diasteromers which are not purified. Removal of the BOC group from
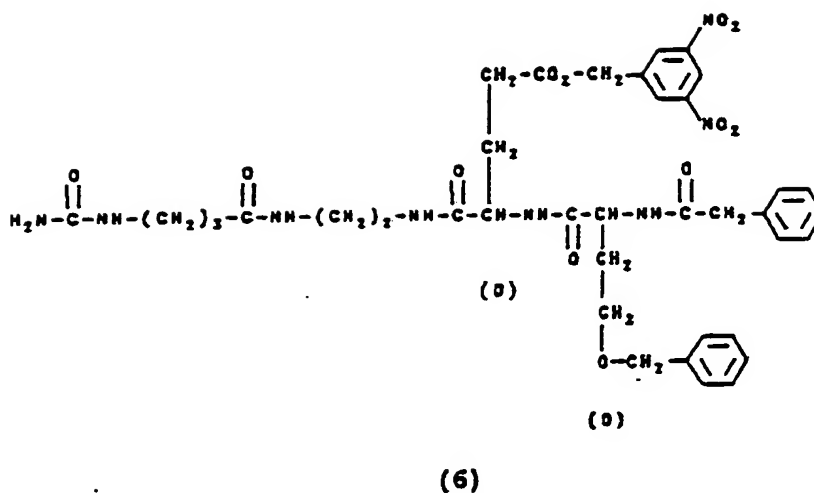
-45-

the piperazine side followed by DCC coupling with the unprotected 5-guanidinylpentanoic acid, above, gives the desired tripeptide (5). Chromatography on a reversed phase C-4 medium pressure column gives the desired compound as a mixture of diastereomers.

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EXAMPLE 7

Synthesis of

(6)

The depicted compound, 4-ureidobutylethylenediamino -D-glutamyl(gamma-3,5-dinitrobenzyl ester)-D-homoserinyl (benzyl)-phenylacetateamide (6) is prepared as follows.

5

4-Ureidobutyric Acid. This intermediate is prepared according to the procedure of V.E. Marquez, J.A. Kelly and J.S. Driscoll, *J. Org. Chem.*, **1980**, 45, 5308.

N'-BOC-ethylenediamine. This compound is prepared according to the procedure of Callahan, *J. Med. Chem.*, **1989**, 32, 341.

10

N-BOC-D-Glutamic Acid (Y-3,5-dinitrobenzyl ester). To N-BOC-D-Glu-α-t-butyl ester (Bachem) dissolved in DMF containing 2 equivalents of triethylamine, 3,5-dinitrobenzyl chloride (Aldrich) is added slowly. After completion of the

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reaction the solvent is removed *in vacuo* and the residue is dissolved in ethyl
acetate. Three equivalents of 4 M HCl is added and after two hours one volume
of ether is added and the N- and C-terminal deprotected D-Glu(gamma-3,5-
dinitrobenzyl ester) collected. The N-terminal nitrogen is reprotected by stirring the

5   D-Glu (gamma-3, 5-dinitrobenzyl ester) and 1 equivalent of di-t-butyl-dicarbonate
(Fluka) in 1:1 dioxane:2N KOH at 0°C overnight. The mixture is concentrated and
extracted thrice with ethyl acetate. The pooled ethyl acetate layers are dried with
sodium sulfate, filtered and concentrated. The desired compound named above
is isolated following purification by column chromatography.

10 BOC-D-Homoserine (Benzyl)-OH. BOC-D-Asp(OBzl)OH (Bachem Biosciences) is
dissolved in ethyl acetate and treated with diazomethane in ethyl ether to produce
the methyl ester. After neutralization of excess diazomethane with acetic acid, the
material is subjected to medium pressure catalytic hydrogenation (Parr) with 5%
Pd/C. The solution is filtered through a Celite mat and concentrated to dryness.

15 The residue is then dissolved in dry THF and treated with one equivalent of
BH_3·dimethyl sulfide complex (Aldrich) [C.F. Lane, *Aldrichimica Acta*, 1975, 8, 20].
On completion of the side chain carboxyl reduction the mixture is diluted with ethyl
acetate. The solution is washed in order with 5% citric acid, 10% sodium
bicarbonate and water, dried and concentrated to dryness. The BOC-homoserine

20 methyl ester is dissolved in DMF and treated first with one equivalent of sodium
hydride, and then benzyl bromide (Aldrich). On completion of the reaction, water
is added to the reaction mixture and the entire mixture evaporated to dryness. The
residue is taken up in wet methanol and treated with threefold excess lithium
hydroxide until the methyl ester hydrolysis is complete. The product is purified by

25 column chromatography to give the title compound.

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4-Ureidobutyl-ethylenediamino-D-glutamyl(gamma-3,5-dinitrobenzyl ester)-D-homoserinyl (benzyl)-phenylaceteamide (6). The 4-ureidobutyric acid and one equivalent of N'-BOC-ethylenediamine from above are condensed with the general DCC coupling procedure described above. The product is deprotected with 4 N HCl in ethyl acetate for 2 hours and neutralized with DIEA. The product is deprotected as usual and coupled with N-BOC-D-Glu (gamma-3, 5-dinitrobenzyl ester), above, using the general DCC method. The product is deprotected and coupled to BOC-D-homoSer(Bzl), above, in an analogous way. This product is deprotected, neutralized, and coupled to the symmetric anhydride of phenylacetic acid (Aldrich). The final purification by reversed phase HPLC and lyophilization affords the desired compound, (6).

★ ★ ★ ★

Purification of the compounds of the invention may be achieved using methods known in the art, including reversed phase HPLC size exclusion chromatography, partition chromatoqraphy on polysaccharide gel media such as Sephadex G10 or G25, or counter current distribution. One potentially important aspect in final purification is the removal of fluoride, which if present in even small amounts may alter the biological profile of the compound. Generally, ion exchange chromatoqraphy, using AG₃-4X acetate for example, to exchange the fluoride salt, may be used. The subject compound is dissolved in water and passed over an acetate-form resin. The eluate is collected, and lyophilized to dryness.

Salts of carboxyl groups of the product compounds may be prepared in the usual manner by contacting the compound with one or more equivalents of a desired base such as, for example, a metallic hydroxide base such as, for example, sodium

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hydroxide; a metal carbonate or bicarbonate base such as, for example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like. In all such procedures and in other synthetic procedures provided herein, the pH should be kept below approximately

5   8 in order to avoid complications such as racemization, deamidation, degradation or other undesirable side reactions.

Acid salts of the compounds may be prepared by contacting the compound with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid, acetic acid or citric acid.

10   Esters or amides of carboxyl groups of the compounds may be prepared by any of the usual means known in the art for converting a carboxylic acid or precursor to an ester or amide.

* * * *

Therapeutic Utility

15   · In the practice of the therapeutic methods of the present invention, an effective amount of the active compound, including derivatives or salts thereof, or a pharmaceutical composition containing the same, as described below, is administered via any of the usual and acceptable methods known in the art, either singly or in combination with another compound or compounds of the present

20   invention or other pharmaceutical agents such as immunosuppressants, antihistamines, corticosteroids, and the like. These compounds or compositions can thus be administered orally, sublingually, topically (e.g., on the skin or in the eyes), parenterally (e.g., intramuscularly, intravenously, subcutaneously or

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intradermally), or by inhalation, and in the form of either solid, liquid or gaseous dosage including tablets, suspensions, and aerosols, as is discussed in more detail below. The administration can be conducted in single unit dosage form with continuous therapy or in single dose therapy ad libitum.

Useful pharmaceutical carriers for the preparation of the pharmaceutical compositions hereof can be solids, liquids or gases; thus, the compositions can take the form of tablets, pills, capsules, powders, enterically coated or other protected formulations (such as by binding on ion exchange resins or other carriers, or packaging in lipid protein vesicles or adding additional terminal amino acids), sustained release formulations, solutions (e.g., ophthalmic drops), suspensions, elixirs, aerosols, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic) for injectable solutions. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical expedients such as sterilization and may contain conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers, and the like. Suitable pharmaceutical carriers and their formulations are described in Martin, *Remington's Pharmaceutical Sciences*, 15th Ed. (Mack Publishing Co., Easton 1975) (see, e.g., pp. 1405-1412, 1461-1487). Such compositions will, in general, contain an effective amount of the

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active compound together with a suitable amount of carrier so as to prepare the proper dosage form for proper administration to the host.

In one preferred embodiment, the therapeutic methods of the present invention are practiced when the relief of symptoms is specifically required or perhaps imminent;

5   in another preferred embodiment, the method hereof is effectively practiced as continuous or prophylactic treatment.

In the practice of the therapeutic methods of the invention, the particular dosage of pharmaceutical composition to be administered to the subject will depend on a variety of considerations including the nature of the disease, the severity thereof,

10   the schedule of administration, the age and physical characteristics of the subject, and so forth. Proper dosages may be established using clinical approaches familiar to the medicinal arts. It is presently believed that dosages in the range of 0.1 to 100 mg of compound per kilogram of subject body weight will be useful, and a range of 1 to 100 mg per kg generally preferred, where administration is by

15   injection or ingestion. Topical dosages may utilize formulations containing generally as low as 0.1 mg of compound per ml of liquid carrier or excipient, with multiple daily applications being appropriate.

The compounds and therapeutic or pharmaceutical compositions of the invention are useful in the study or treatment of diseases or other conditions which are

20   mediated by the binding of integrin receptors to ligands, including conditions involving inappropriate (i.e., excessive or insufficient) binding of cells to natural or other ligands. Such diseases and conditions include inflammatory diseases such as rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases (e.g., ulcerative colitis and regional

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enteritis) and opthalmic inflammatory diseases; autoimmune diseases; thrombosis or inappropriate platelet aggregation conditions; reocclusion following thrombolysis; cardiovascular disease; neoplastic disease including metastasis conditions; as well as conditions wherein increased cell binding is desired, as in

5   wound healing or prosthetic implantation situations as discussed in more detail above. Examples of "excessive" binding of cells to ligands include conditions, such as metastasis, where particular cell binding is inappropriate to the health of the subject and is sought to be eliminated to the maximum extent possible.

In addition, derivatives of the present compounds may be useful in the generation

10   of antigens which, in turn, may be useful to generate antibodies. These antibodies will, in some cases, themselves be effective in inhibiting cell adhesion or modulating immune activity by acting as receptors for matrix proteins or other cell adhesion ligands, or, if antiidiotypic, by acting to block cellular receptors.

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EXAMPLE 8

Cell Adhesion Inhibition Assay

The following assay established the activity of the present compounds in inhibiting cell adhesion in a representative *in vitro* system. The assay was a competition

5   assay in which both fibronectin and a test compound were present. Microtiter plates were first precoated with fibronectin. The test compound was then added in increasing concentrations with cells known to contain the fibronectin receptor. The plates were then washed and stained for quantitation of attached cells. The present assay directly demonstrates the anti-cell adhesion activity and modulatory

10   activity of the present compounds. For example, by immobilizing the compound on a surface, one could adhere appropriate cells to that surface. Other cell adhesion modulation activity, and utilities pertinent thereto, will be apparent to those skilled in the art.

The cell line U937 was purchased from American Type Tissue Culture Collection.

15   The cells were cultured in RPMI media (J.R. Scientific Company, Woodland Hills, California 95695) containing 10% fetal calf serum. Fibronectin was purified from human plasma according to the procedure of Engvall, E. and Ruoslahti, E., *Int. J. Cancer* 1977, 20, 1-4.

Microtiter plates (96-well, Falcon) were coated overnight at 4°C with 5 μg/ml

20   fibronectin (FN) (for a total volume of 0.1 ml) or, as a control, 5 μg/ml bovine serum albumin (BSA) diluted in phosphate buffered saline (PBS, 0.01 M $NaPO_4$ in 0.9% NaCl at pH 7.2 to 7.4). Unbound proteins were removed from plates by washing with PBS. The plates were then coated With 100 μl of PBS containing 2.5 mg/ml BSA for one hour at 37°C. This procedure is a modification of a

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previously published method, Cardarelli, P.M. and M.D. Pierschbacher, *PNAS-USA*, 1986, 83, 2647-2651. The containment in the wells of functional amounts of immobilized protein has been confirmed by independent assay of fibroblast attachment and ELISA (Engvall, E., *Methods Enzymol.*, 1980, 70, 419-439), although the actual amount of protein bound to the plate in these assays was not determined.

A U937 culture was collected and washed two times with Hanks Balanced Salt Solution. The cells were counted and adjusted to 1.5×10^6 cells per ml in Dulbecco's Modified Eagles Medium (DMEM) plus BSA (2.5 mg/ml) for cell attachment assay. Subject compounds were then dissolved in DMEM and BSA, and the pH was adjusted to 7.4 with 7.5% sodium bicarbonate. The compounds (100 μl) were added to FN-coated wells, at 1.5, 0.75, 0.375, 0.188, 0.094, 0.047, 0.023, 0.012, 0.006 and 0.003 mg/ml final concentration and U937 cells (100 μl) were added per well. The plates were then incubated at 37°C for 60 minutes. Following this incubation, the plates were washed once with PBS. Attached cells were fixed with 3% paraformaldehyde in PBS and stained with 0.5% toluidine blue in 3.7% formaldehyde. The cells were stained overnight at room temperature and the optical density at 590 nm of toluidine blue-stained cells was determined using a vertical pathway spectrophotometer to quantitate attachment (VMAX Kinetic Microplate Reader, Molecular Devices, Menlo Park, California 94025).
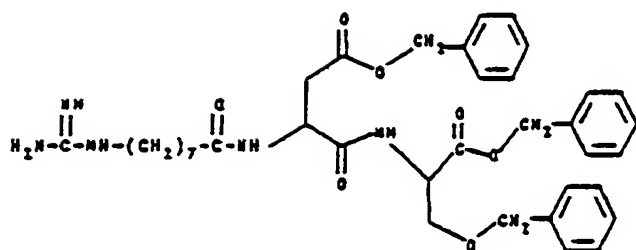
-55-

Results. The table below shows the results of the cell adhesion inhibition assay for representative compounds of the invention. Potency is expressed in μM units. The accompanying figure is a diagram representing the curve of cell adhesion inhibition for the compound
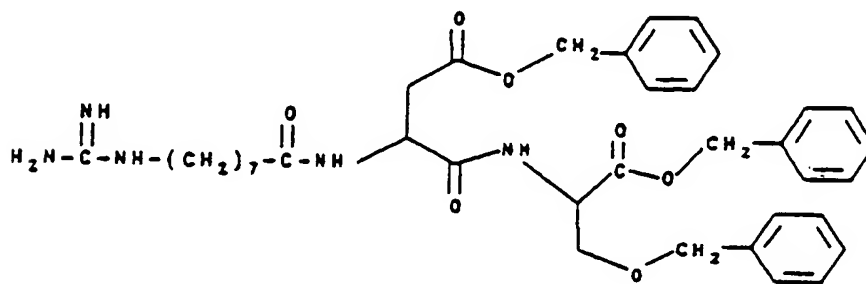
ACTIVITY OF COMPOUNDS IN THE U937
FIBRONECTIN ADHESION ASSAY

Peptide	IC_{50}, μM

345

$$\underset{\overset{\displaystyle \parallel}{NH}}{H_2N-C-NH-(CH_2)_3-\overset{\overset{\displaystyle O}{\parallel}}{C}-Gly-Asp-Ser}$$

2400

$$\underset{\overset{\displaystyle \parallel}{NH}}{H_2N-C-NH-(CH_2)_5-\overset{\overset{\displaystyle O}{\parallel}}{C}-Gly-Asp-Ser}$$

2500

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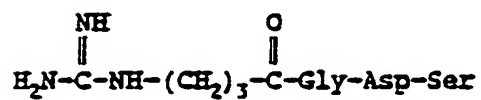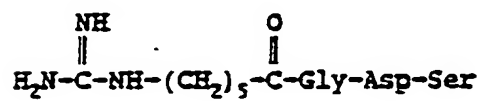
In addition, the compounds

$$\overset{\text{NH}}{\underset{\parallel}{}} \quad \overset{\text{O}}{\underset{\parallel}{}}$$
$$H_2N\text{-}C\text{-}NH\text{-}(CH_2)_2\text{-}C\text{-}Gly\text{-}Asp\text{-}Ser \quad \text{and}$$

$$\overset{\text{NH}}{\underset{\parallel}{}} \quad \overset{\text{O}}{\underset{\parallel}{}}$$
$$H_2N\text{-}C\text{-}NH\text{-}(CH_2)_4\text{-}C\text{-}Gly\text{-}Asp\text{-}Ser$$
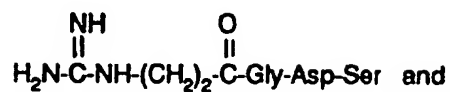
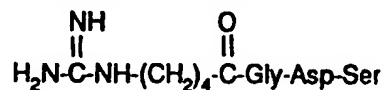were also synthesized and tested in the cell adhesion inhibition assay described above. Specific activity levels were not established inasmuch as the IC_{50} of the compounds was determined to be in excess of 1.5 mg/m· Thus, although such compounds are believed to be active as cell adhesion modulators at higher dosage levels, they are presently not as highly preferred as the compounds exemplified above.
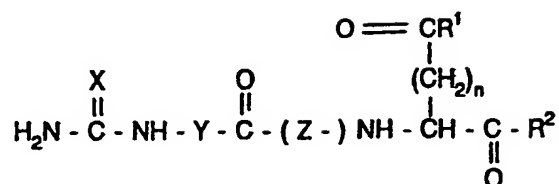
The foregoing examples are given to enable those skilled in the art to more fully understand and practice the present invention. They should not be construed as a limitation upon the scope of the invention, which is set forth in the appended claimg, but merely as being illustrative and representative thereof.

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CLAIMS

1.   A compound of the formula

$$O = \underset{\underset{\displaystyle H_2N - \underset{\parallel}{\overset{\parallel}{C}} - NH - Y - \underset{\parallel}{\overset{\parallel}{C}} - (Z-) NH - \underset{\displaystyle \underset{\parallel}{\overset{\parallel}{C}} - R^2}{\overset{\displaystyle (CH_2)_n}{CH}}}{\underset{\displaystyle O}{}}}{\underset{}{\overset{\displaystyle CR^1}{}}}$$

and pharmaceutically acceptable salts thereaf, wherein

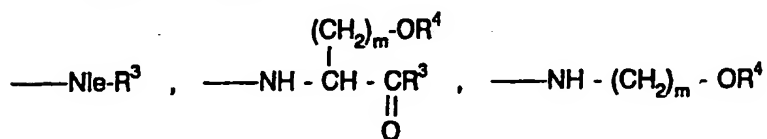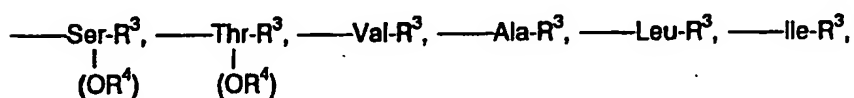X   is selected from NR, S and O;

Y   is an unsubstituted or substituted, linear or branched linking moiety
       selected from saturated and unsaturated hydrocarbon groups
       containing from 1 to about 15 linking atoms,

Z   is optional and, where present, is selected from Gly, Ala, Sar and
       β-Ala;

R^1 and R^2 are independently substituents selected from -OR (including
       hydroxyl), $-NR_2$ (including $-NH_2$ and -NHR), $-NHNH_2$ and -SR, and
       where one of $R^1$and R^2 may additionally be selected from
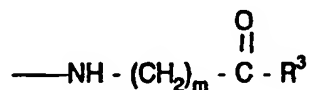
$$\underset{(OR^4)}{—Ser\text{-}R^3,} \quad \underset{(OR^4)}{—Thr\text{-}R^3,} \quad —Val\text{-}R^3, \quad —Ala\text{-}R^3, \quad —Leu\text{-}R^3, \quad —Ile\text{-}R^3,$$

$$—Nle\text{-}R^3 \quad , \quad —NH - \underset{\underset{\displaystyle O}{\parallel}}{\overset{\displaystyle (CH_2)_m\text{-}OR^4}{CH} - CR^3} \quad , \quad —NH - (CH_2)_m - OR^4$$

and

$$—NH - (CH_2)_m - \overset{\displaystyle O}{\underset{\parallel}{C}} - R^3$$
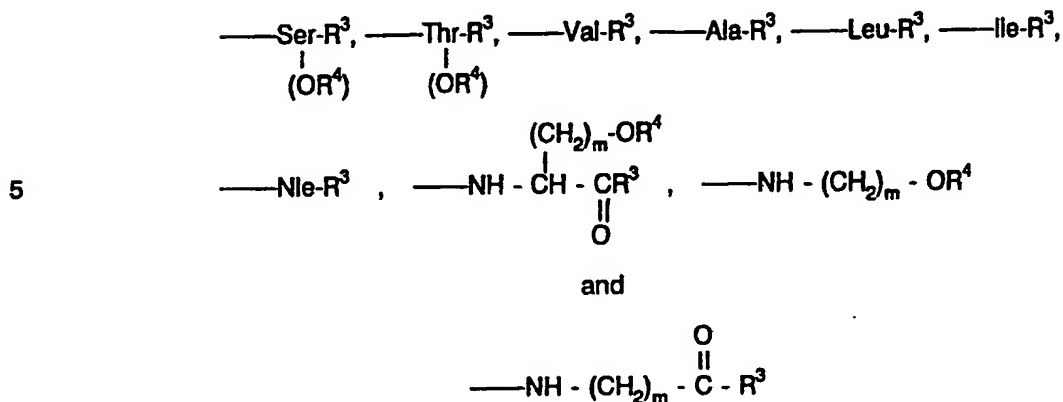
wherein R^3 is a substituent selected from -OR

25

　　　　(including hydroxyl), $-NR_2$ (including $-NH_2$ and -NHR), $-NHNH_2$ and -SR, and

R^4　　Is a substituent selected from groups of the form -R and acyl groups -COR;

n　　and (where present) m are independently 1, 2 or 3; and wherein

30

　　　　each R group is individually a pharmaceutically suitable substituent group selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} aralkyls and C_3-C_{14} cycloalkyls, from heteroatomic groups and, in the case of $-NR_2$, from

35

　　　　cyclized groups forming (in attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.
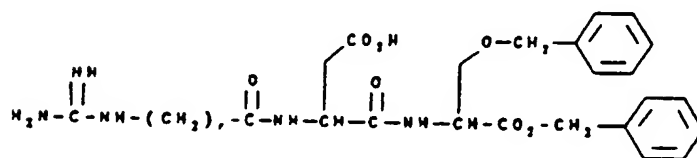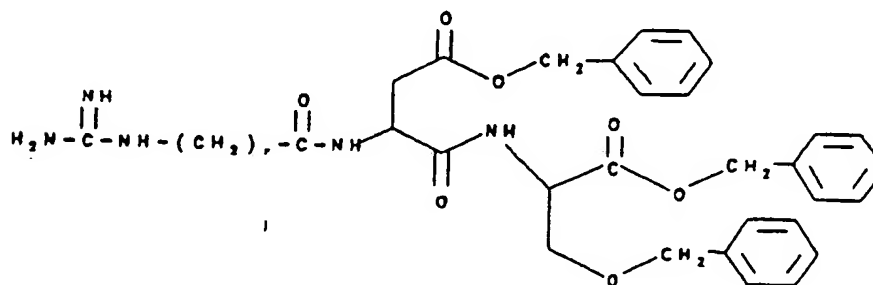
2.　　The compound of claim 1 wherein X is HN.

3.　　The compound of claim 1 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

4.　　The compound of claim 3 wherein each R group is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties.
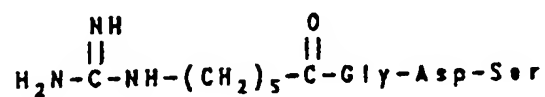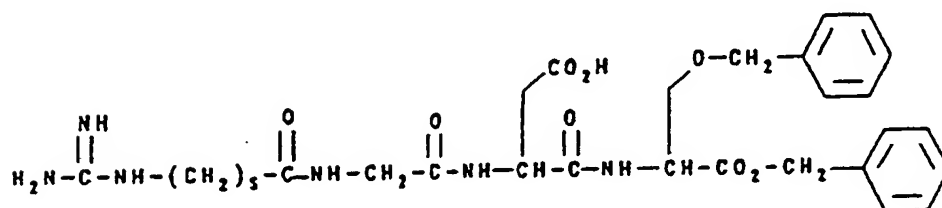
-60-

5. The compound of claim 1 wherein one or more R group is selected from heterocyclic groups.

6. The compound of claim 1 wherein one of R^1 and R^2 is selected from

$$—Ser-R^3, \quad —Thr-R^3, \quad —Val-R^3, \quad —Ala-R^3, \quad —Leu-R^3, \quad —Ile-R^3,$$
$$\quad\quad\;\; |(OR^4) \quad\quad\;\; |(OR^4)$$

$$—Nle-R^3 , \quad —NH - \underset{\underset{O}{\overset{\parallel}{C}R^3}}{\overset{(CH_2)_m-OR^4}{\overset{|}{CH}}} , \quad —NH - (CH_2)_m - OR^4$$

and

$$—NH - (CH_2)_m - \overset{\overset{O}{\parallel}}{C} - R^3$$

7. The compound of claim 6 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

8. The compound of claim 6 wherein at least one R group is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties.
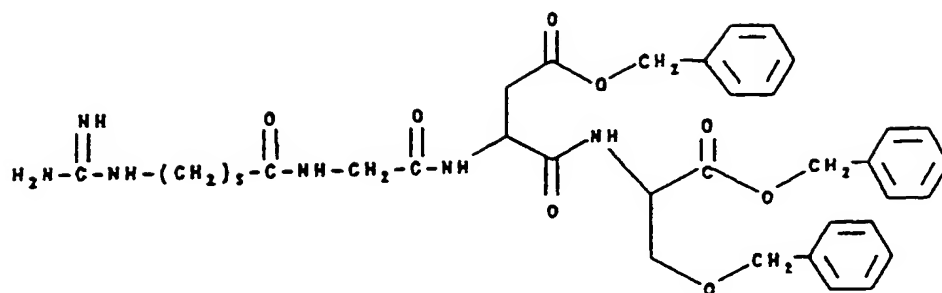
9. The compound of claim 6 wherein R^1 and R^3 are of the form -OR.

-61-

10. The compound of claim 9 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

11. The compound of claim 9 wherein each R group in R^3 and, if present, R^4 is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties.
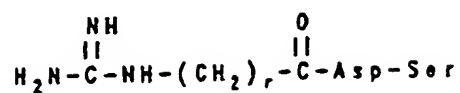
12. The compound of claim 1 wherein Y is a hydrocarbon chain from 1 to about 8 linking atoms.

13. The compound of claim 6 wherein Y is a hydrocarbon chain from 1 to about 8 linking atoms.

14. The compound of claim 1 wherein the total number of linking atoms L^{tot} is from about 9 to about 12.

15. A compound of claim 1 selected from the group consisting of

$$\underset{\substack{\displaystyle \| \\ \displaystyle H_2N-C-NH-(CH_2)_r-C-Asp-Ser}}{\overset{\substack{NH \qquad\qquad O \\ \| \qquad\qquad \|}}{}}$$

$$\underset{\substack{H_2N-C-NH-(CH_2)_s-C-Gly-Asp-Ser}}{\overset{\substack{NH \qquad\qquad O \\ \| \qquad\qquad \|}}{}}$$
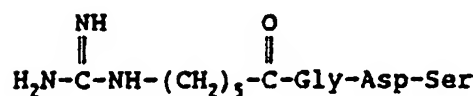
and pharmaceutically acceptable salts thereof, where r is an integer of from
1 to about 8 and s is an integer of from 1 to about 5.

-63-

16.   A compound of claim 14 selected from the group consisting of

$$\begin{array}{c} NH \\ \parallel \\ H_2N-C-NH-(CH_2)_3- \end{array} \begin{array}{c} O \\ \parallel \\ C \end{array} -Gly-Asp-Ser$$

$$\begin{array}{c} NH \\ \parallel \\ H_2N-C-NH-(CH_2)_5- \end{array} \begin{array}{c} O \\ \parallel \\ C \end{array} -Gly-Asp-Ser$$
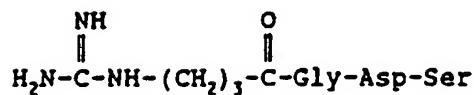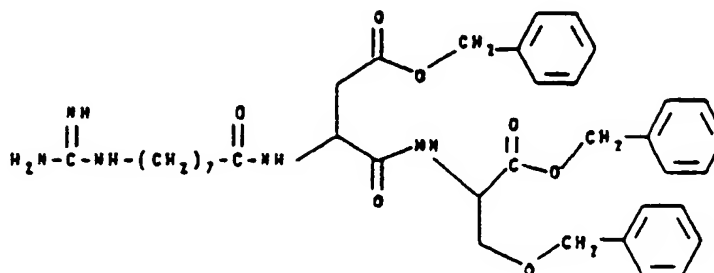
and pharmaceutically acceptable salts thereof.

17.   A compound of the formula

$$\begin{array}{c} O=CR^1 \\ \quad | \\ \begin{array}{cc} X & O \;\; (CH_2)_n \\ \parallel & \parallel \quad | \end{array} \\ H_2N-C-NH-Y-(Z'-)\,C-CH-NRR^5 \end{array}$$
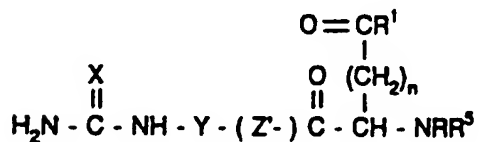
and pharmaceutically acceptable salts thereof, wherein

X   is selected from NR, S and O;

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Y is an unsubstituted or substituted, linear or branched linking moiety selected from saturated and unsaturated · hydrocarbon groups containing from 1 to about 15 linking atoms

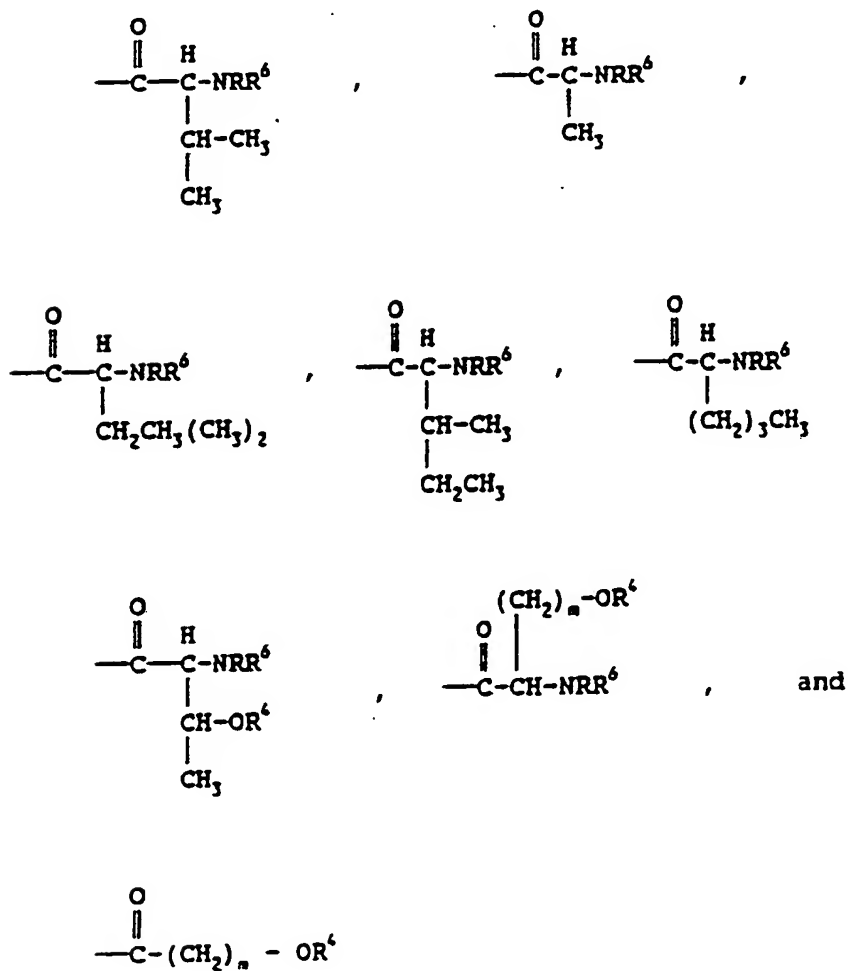10 R^1 is a substituent selected from $-OR$ (including hydroxyl), $-NR_2$ (including $-NH_2$ and $-NHR$), $-NHNH_2$ and $-SR$;

R^5 is selected from $-R$, acyl groups $-COR$, alkyloxycarbonyl groups of the form $-\underset{\underset{O}{\|}}{C}-OR$,

15 and from groups of the form

$$-\underset{\underset{\underset{\underset{CH_3}{|}}{CH-CH_3}}{|}}{\overset{\overset{O}{\|}}{C}}-\underset{H}{\overset{H}{C}}-NRR^6 \qquad , \qquad -\underset{\underset{CH_3}{|}}{\overset{\overset{O}{\|}}{C}}-\overset{H}{C}-NRR^6 \qquad ,$$

$$-\underset{\underset{CH_2CH_3(CH_3)_2}{|}}{\overset{\overset{O}{\|}}{C}}-\overset{H}{C}-NRR^6 \qquad , \qquad -\underset{\underset{\underset{\underset{CH_2CH_3}{|}}{CH-CH_3}}{|}}{\overset{\overset{O}{\|}}{C}}-\overset{H}{C}-NRR^6 \qquad , \qquad -\underset{\underset{(CH_2)_3CH_3}{|}}{\overset{\overset{O}{\|}}{C}}-\overset{H}{C}-NRR^6$$

$$-\underset{\underset{\underset{\underset{CH_3}{|}}{CH-OR^6}}{|}}{\overset{\overset{O}{\|}}{C}}-\overset{H}{C}-NRR^6 \qquad , \qquad -\underset{\underset{(CH_2)_n-OR^6}{|}}{\overset{\overset{O}{\|}}{C}}-CH-NRR^6 \qquad , \qquad and$$

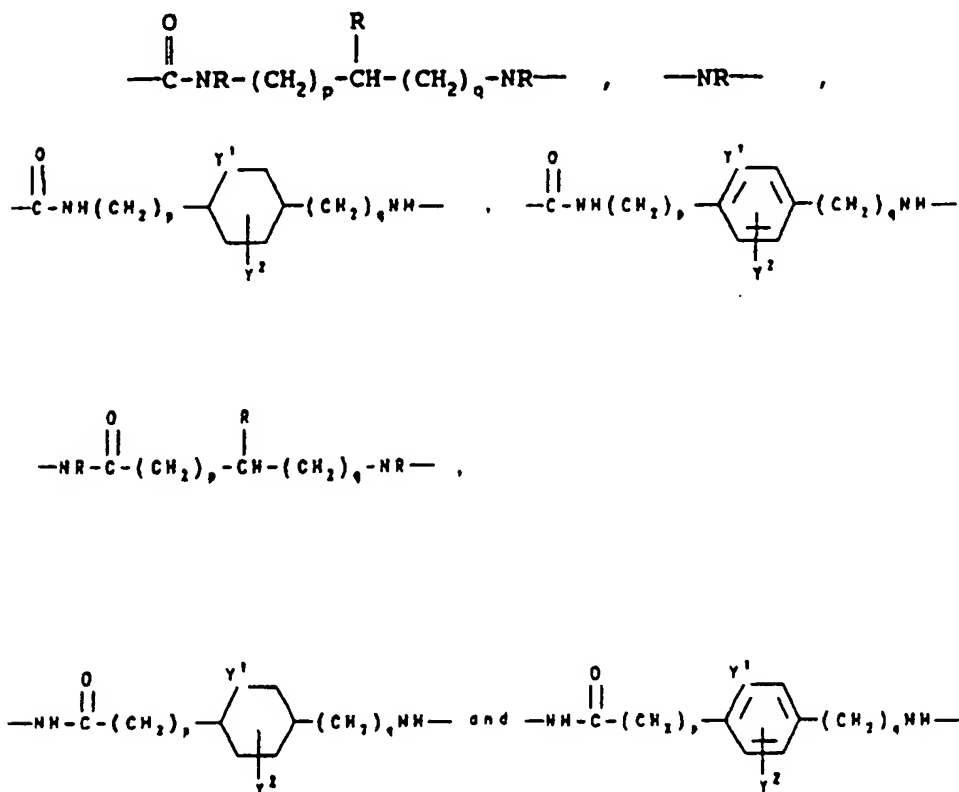$$-\underset{}{\overset{\overset{O}{\|}}{C}}-(CH_2)_n - OR^6$$

-65-

wherein R^4 and R^6 are substituents independently selected from groups of the form -R and acyl groups -COR, and R^6 may additionally be selected from alkoxycarbonyl groups of the form $-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}$-OR; Z' is a linking moiety

20   selected from those of the form

$$-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{-NR-}(CH_2)_p\text{-}\overset{R}{\underset{|}{\text{CH}}}\text{-}(CH_2)_q\text{-NR}— \quad , \qquad —NR— \quad ,$$

$$-\overset{\text{O}}{\underset{\text{||}}{\text{NR-C}}}-(CH_2)_p\text{-}\overset{R}{\underset{|}{\text{CH}}}-(CH_2)_q\text{-NR}— \quad ,$$

wherein

Y^1 comprises carbon or an O, S or N heteroatom;

Y^2 is an optional substituent selected from hydroxyl, amino, lower $(C_1\text{-}C_6)$ alkoxyl and alkyl, nitro and halogen moieties;

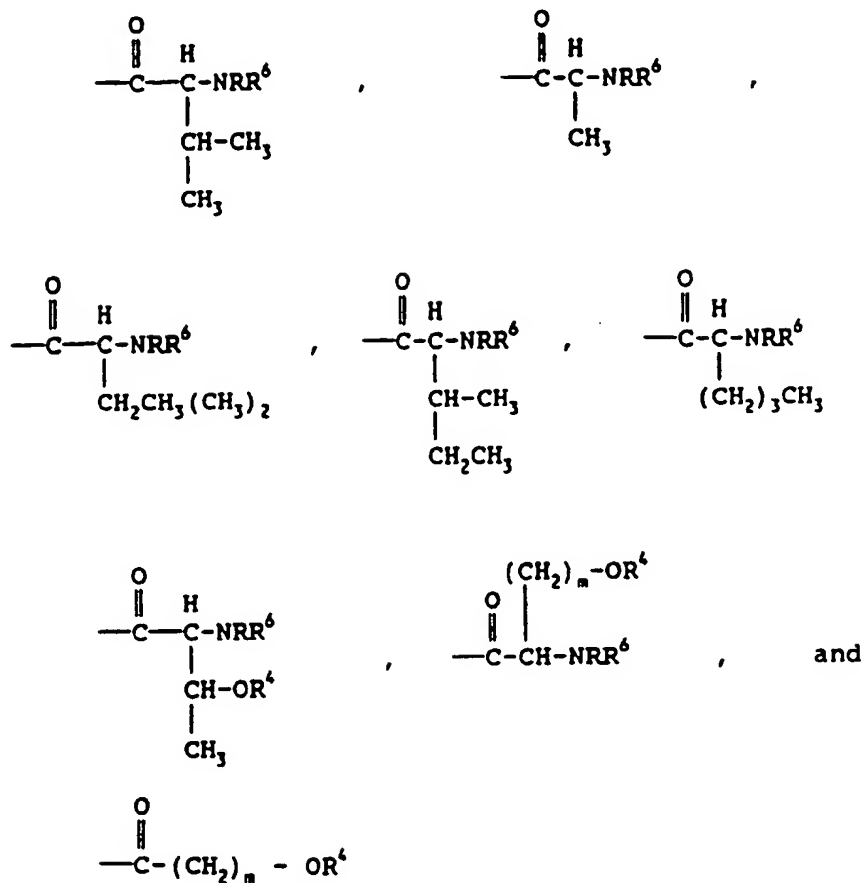25   p and q are independently integers of from 0 to about 5;

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n and (where present) m are Independently 1, 2 or 3; and wherein each R group is individually a pharmaceutically suitable substituent group selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_6 lower alkyls, C_2-C_6 alkenyls, C_2-C_6 alkynyls, C_6-C_{14}aryls, C_7-C_{14} alkaryls, C_7-C_{14} aralkyls and C_3-C_{14} cycloalkyls, from heteroatomic groups and, in the case of -NR$_2$ from cyclized groups forming (in attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

18.   The compound of claim 17 wherein X is HN.

19.   The compound of claim 17 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

20.   The compound of claim 19 wherein each R group is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties.

21.   The compound of claim 17 wherein one or more R group is selected from heterocyclic groups.

22. The compound of claim 17 wherein R^5 is selected from groups of the form

$$
\underset{\substack{| \\ CH-CH_3 \\ | \\ CH_3}}{-C}\overset{\overset{O}{\|}}{}\overset{H}{\underset{|}{C}}-NRR^6 \quad , \qquad -\overset{\overset{O}{\|}}{C}-\overset{H}{\underset{\substack{| \\ CH_3}}{C}}-NRR^6 \quad ,
$$

$$
-\overset{\overset{O}{\|}}{C}-\overset{H}{\underset{\substack{| \\ CH_2CH_3(CH_3)_2}}{C}}-NRR^6 \quad , \qquad -\overset{\overset{O}{\|}}{C}-\overset{H}{\underset{\substack{| \\ CH-CH_3 \\ | \\ CH_2CH_3}}{C}}-NRR^6 \quad , \qquad -\overset{\overset{O}{\|}}{C}-\overset{H}{\underset{\substack{| \\ (CH_2)_3CH_3}}{C}}-NRR^6
$$

$$
-\overset{\overset{O}{\|}}{C}-\overset{H}{\underset{\substack{| \\ CH-OR^4 \\ | \\ CH_3}}{C}}-NRR^6 \quad , \qquad -\overset{\overset{O}{\|}}{C}-\overset{\overset{\textstyle (CH_2)_n-OR^4}{|}}{CH}-NRR^6 \quad , \qquad \text{and}
$$

$$
-\overset{\overset{O}{\|}}{C}-(CH_2)_n - OR^4
$$

23. The compound of claim 22 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

24. The compound of claim 22 wherein at least one R group is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties.

25. The compound of claim 22 wherein R^1 and R^6 are of the form -OR or, in the case of R^6, of the form $-\overset{\text{O}}{\underset{\text{}}{\overset{\|}{C}}}$-OR.

26. The compound of claim 25 wherein each R group is independently selected from hydrogen and from unsubstituted and substituted lower alkyl and single-ring aryl hydrocarbon moieties.

27. The compound of claim 25 wherein each R group in R^6 and, if present, R^4 is independently selected from unsubstituted and substituted single-ring aryl hydrocarbon moieties .

28. The compound of claim 17 wherein Y is a hydrocarbon linking moiety of from 1 to about 8 linking atoms.

29. The compound of claim 22 wherein Y is a hydrocarbon chain of from 1 to about 8 linking atoms.

30. The compound of claim 17 wherein the total number of linking atoms L^{tot} is from about 9 to about 12.

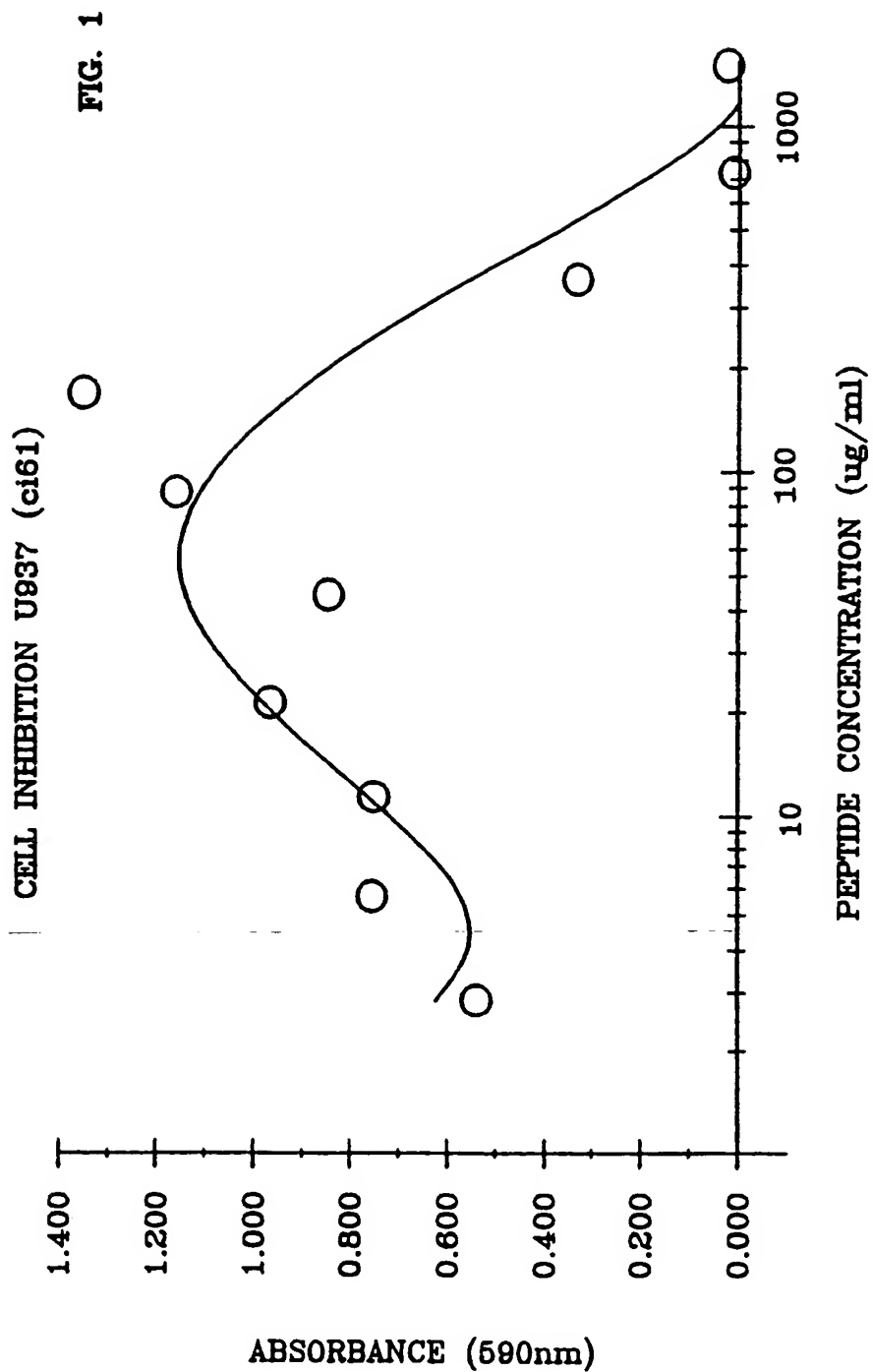31. A compound of claim 17 selected from the group consisting of:
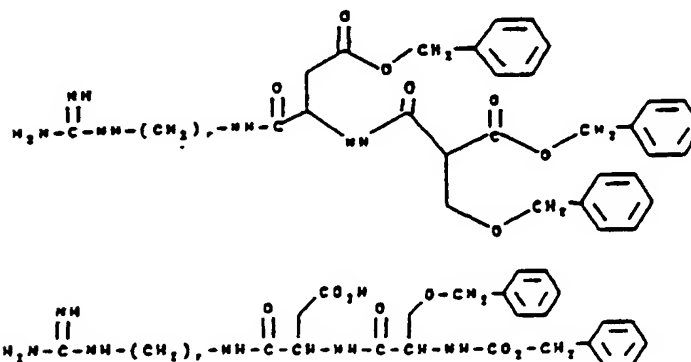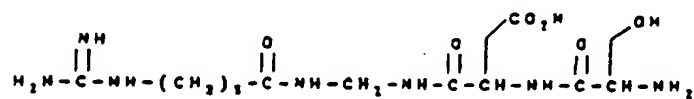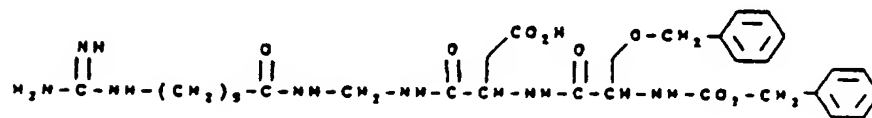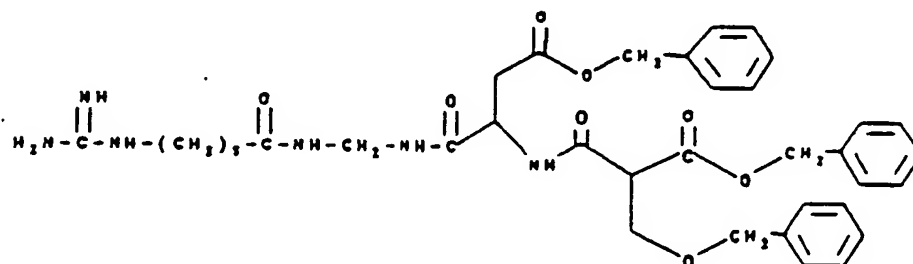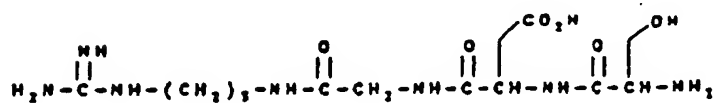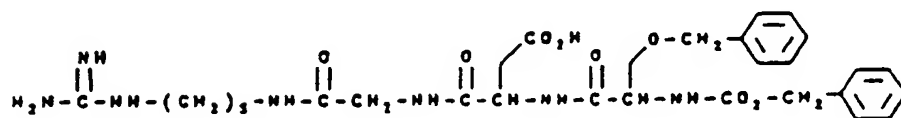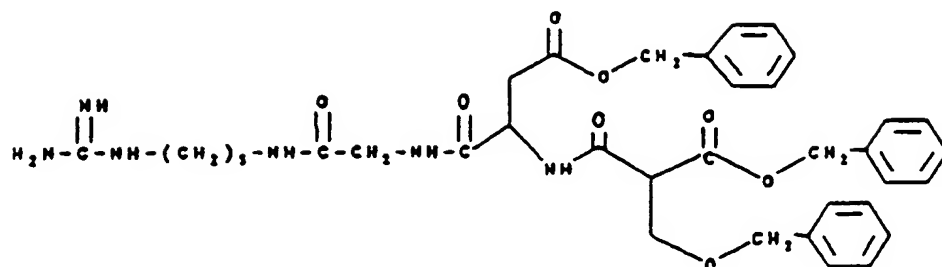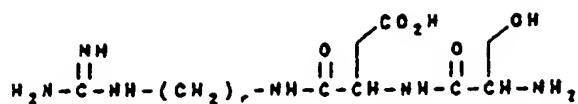
and pharmaceutically acceptable salts thereof, where r is an integer of from 1 to about 8 and s is an integer of from 1 to about 5.

32. A compound of claim 1 useful in modulating adhesion of a cell expressing a fibronectin receptor.

33. A compound of claim 17 useful in modulating adhesion of a cell expressing a fibronectin receptor.

34. A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 1.

35. The method of claim 34 wherein said cells express an integrin receptor.

36. The method of claim 34 wherein said cells express a receptor for fibronectin.

37. The method of claim 34 wherein said system is a mammalian system characterized by an excessive or inadequate level of cell adhesion.

38. A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 1, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, reocclusion following thrombolysis,

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cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions.

39.   A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 17.

40.   The method of claim 39 wherein said cells express an integrin receptor.

41.   The method of claim 39 wherein said cells express a receptor for fibronectin.

42.   The method of claim 39 wherein said system is a mammalian system characterized by an excessive or inadequate level of cell adhesion.

43.   A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 17, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, reocclusion following thrombolysis, cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions.

44.   A pharmaceutical composition comprising a compound of claim 1.

45.   A pharmaceutical composition comprising a compound of claim 17.

FIG. 1

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02; C07K 5/00, 5/06, 5/08
USC1.: 514/18,20; 530/331

II. FIELDS SEARCHED

	Minimum Documentation Searched [7]
Classification System	Classification Symbols
U.S.C1.	514/18,20; 530/331

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

CHEMICAL ABSTRACTS SERVICE, MEDLINE, AND BIOSIS DATABASES

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A,E	US,A, 5,061,693 (NUTT et al.) 29 October 1991.	1-45
A,E	US,A, 5,053,393 (Tjoeng et al.) 01 October 1991.	1-45
A,E	US,A, 5,053,392 (Klein et al.) 01 October 1991.	1-45
X	US,A, 4,879,313 (Tjoeng et al.) 07 November 1989. See especially col. 2, lines 19-36.	1-5,12-14, 32,34-38, and 44
A	US,A, 4,952,562 (Klein et al.) 28 August 1990.	1-45

* Special categories of cited documents: [10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
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ISA/US	Susan M. Perkins

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(54) Title: AMORPHOUS SALTS OF ROSUVASTATIN
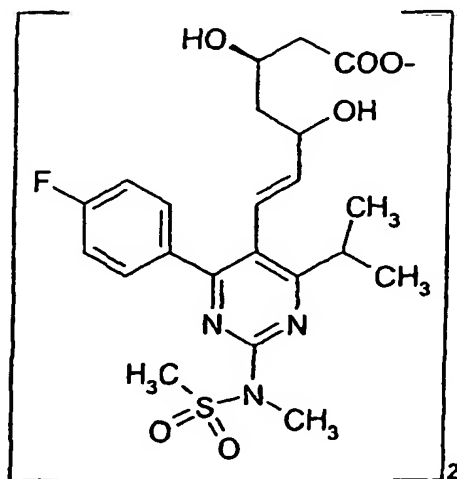
(57) Abstract: The invention provides the novel
amorphous rosuvastatin magnesium and process
for preparation thereof from crystalline rosuvastatin
magnesium, rosuvastatin methyl ammonium
salt and from rosuvastatin lactone. Rosuvastatin
magnesium is, chemically, (3R,5S,6E)-7-[4-(4-flu-
orophenyl)-6-(1-methylethyl)-2-[methyl(methyl-
sulfonyl)amino]-5-pyrimidinyl]-3,5-dihy-
droxy-6-heptenoic acid, magnesium salt (2:1)
of Formula (I). Rosuvastatin magnesium is an
antihypercholesterolemic drug used in the treatment
of atherosclerosis.

WO 2005/077917 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMORPHOUS MAGNESIUM SALTS OF ROSUVASTATIN

Field of the Invention

This invention relates to amorphous salts of HMG CoA reductase inhibitors, and in particular, amorphous rosuvastatin magnesium, and processes for preparation thereof from

5   crystalline rosuvastatin magnesium, rosuvastatin methyl ammonium salt and from rosuvastatin lactone.

Background of the Invention

Rosuvastatin magnesium is, chemically, (3R,5S,6E)-7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic

10   acid, magnesium salt (2:1) of Formula I.
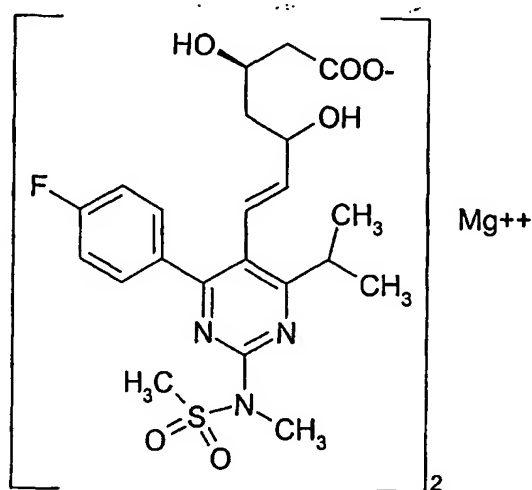
FORMULA I

Rosuvastatin magnesium is an antihypercholesterolemic drug used in the treatment of atherosclerosis.

15   The difference in the activity of different polymorphic forms of a given drug has drawn the attention of many workers in recent years to undertake the studies on polymorphism. This has especially become very interesting since many antibiotics, antibacterials, tranquilizers exhibit polymorphism and particular polymorphic forms of a given drug can exhibit superior bioavailability and consequently show much higher

- 2 -

activity compared to other forms. The term polymorphism includes different physical forms, crystal forms, and crystalline / liquid crystalline / non-crystalline (amorphous) forms.

5   It has also been disclosed that the amorphous forms in a number of drugs can exhibit different dissolution characteristics and in some cases, different bioavailability patterns compared to the crystalline form [Konne T., *Chem. Pharm. Bull.*, 38, 2003 (1990)]. For some therapeutic indications one bioavailability pattern may be favoured over another. Cefuroxime axetil is a classical example of an amorphous form exhibiting higher bioavailability than a crystalline form.

10   US Patent No. RE37314 describes a process for preparation of amorphous rosuvastatin calcium by dissolving the corresponding sodium salt in water, adding calcium chloride, and collecting the resultant precipitate by filtration.

US Patent No. 6,589,959 describes a process for preparation of crystalline form A of rosuvastatin by warming the amorphous form of rosuvastatin calcium in a mixture of

15   water and acetonitrile, cooling the resultant solution to ambient temperature and then filtering the product which is then dried at 50°C under vacuum to give crystalline Form A of rosuvastatin calcium.

The preparation of crystalline rosuvastatin magnesium salt is described in PCT patent application WO 01/60804 from rosuvastatin methyl ammonium salt of Formula II.

20   Rosuvastatin methyl ammonium salt is treated with sodium hydroxide in water to get free rosuvastatin acid, which is then reacted, with magnesium sulphate to get rosuvastatin magnesium salt, which is then isolated and dried. The dried magnesium salt is then heated with water and after diluting the reaction mass with water; the resultant solution is then allowed to stand for 66 hours followed by filtration of the crystalline

25   magnesium salt.

Amorphous rosuvastatin salts can be more effective pharmaceutically than the corresponding crystalline forms. Amorphous forms can be very difficult to isolate and can pose problems with respect to purity and impurity profile. Amorphous forms can also

- 3 -

have stability issued and can convert to more stable crystalline forms by absorption of moisture from atmosphere.

Summary of the Invention

The present inventors have set out to prepare heretofore unknown amorphous rosuvastatin magnesium having a high purity. In one aspect, stable amorphous rosuvastatin magnesium is provided.

It has been discovered that amorphous rosuvastatin magnesium when made by the processes of the present invention is easy to isolate and handle, thus making the process amenable for commercial scale use. The purity of the amorphous rosuvastatin magnesium of the present invention is greater than 99%, with diastereomeric impurity less than 0.5%.

Brief Description of the Drawings

Figure 1 is an X-ray powder diffraction (XRD) pattern of amorphous rosuvastatin magnesium.

Figure 2 is an X-ray powder diffraction (XRD) pattern of crystalline rosuvastatin magnesium.

Figure 3 is an X-ray powder diffraction (XRD)` pattern of a mixture of largely amorphous mixed with some crystalline rosuvastatin magnesium.

Figure 4 is an X-ray powder diffraction (XRD) pattern of amorphous rosuvastatin calcium.

Figure 5 is an X-ray powder diffraction (XRD) pattern of crystalline rosuvastatin calcium.

In one aspect, amorphous rosuvastatin magnesium of Formula I is provided.

The purity of the amorphous form is greater than 99%, with diastereomeric impurity less than 0.5%. For example, the purity is greater than 99.5%, with diastereomeric impurity less than 0.25%, or for example the purity is greater than 99.8%, with diastereomeric

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impurity less than 0.15%. Amorphous rosuvastatin magnesium substantially free of crystalline rosuvastatin magnesium is also provided herein.

In another aspect, an improved process for the preparation of crystalline rosuvastatin magnesium from rosuvastatin methyl ammonium salt or from rosuvastatin
5   lactone is provided. The process comprises

a)   treating rosuvastatin methyl ammonium salt or rosuvastatin lactone with a base and magnesium salt.

b)   isolating crystalline rosuvastatin magnesium from the reaction mass.

Rosuvastatin methyl ammonium salt of Formula II
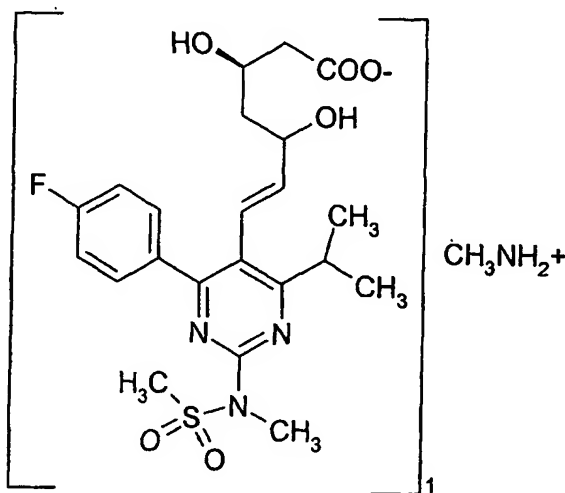
FORMULA II

can be prepared by the process described in PCT application WO 01/60804. Rosuvastatin methyl ammonium salt can be treated with a base in presence of water optionally containing an organic solvent. The reaction mass can then be partially concentrated to
15   remove excess methylamine or ammonia liberated. The reaction mass can then be treated with a compound capable of generating magnesium ions, at a temperature of about 20 to 45°C under stirring. The solution can then finally be cooled to room temperature and filtered to get crystalline rosuvastatin magnesium. The entire process can be carried out in-situ without isolating any other salt of rosuvastatin, such as sodium or calcium. As per the

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process described in Example 9 of PCT application WO 01/60804, the product obtained
can further be crystallized from water, which generally involves 4 hours of stirring, and
about 66 hours of standing, followed by filtration. However, there is no need to further
purify the crystalline rosuvastatin magnesium obtained by the process provided herein.

5   Thus, the process of present invention is less time-consuming and simple to operate on a
commercial scale.

Alternatively, rosuvastatin lactone of Formula III,
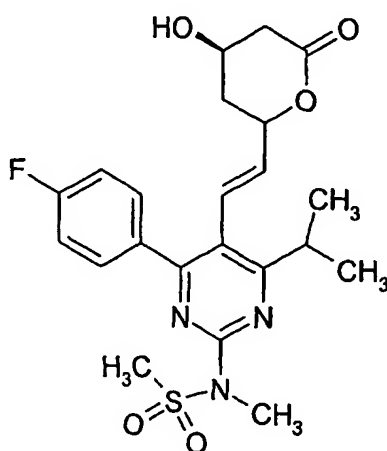
FORMULA III

10   is treated with a base in the presence of water optionally containing an organic solvent, at
a temperature of about 20 to 45°C till hydrolysis of lactone is completed. The organic
solvent can be then completely or partially removed. The resultant reaction mass can then
be treated with a compound capable of generating magnesium ions at a temperature of
about 20 to 45°C under stirring. The solution can then finally be cooled to room

15   temperature and filtered to get crystalline rosuvastatin magnesium. The entire process can
be carried out in-situ without isolating any other salt of rosuvastatin, such as sodium or
calcium.

The crystalline rosuvastatin magnesium obtained can have a purity above about
99% with diastereomeric impurities less than about 0.5%, for example purity above

20   99.25% with diastereomeric impurities less than 0.25%. The crystalline material can be

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used as such for the purpose of preparing medicament and also for the preparation of amorphous rosuvastatin magnesium or amorphous rosuvastatin calcium.

The base is selected from, for example, sodium hydroxide, sodium carbonate, sodium bicarbonate, potassium hydroxide, potassium carbonate or potassium bicarbonate.

5   The magnesium ions can be generated by using a magnesium compound selected from, for example, magnesium chloride, magnesium hydroxide, magnesium carbonate, magnesium acetate, magnesium sulphate, magnesium borate, magnesium tartarate, magnesium bromide or any other compound capable of generating magnesium ions.

The organic solvent can comprise lower alkanols, ethers, esters, ketones, polar
10   aprotic solvents, alkyl or cycloalkyl hydrocarbons or mixtures thereof. The lower alkanol can be, for example, methanol, ethanol, isopropanol, isobutanol, n-butanol and n-propanol. The ethers can be, for example, tetrahydrofuran, 1,4-dioxane, diethyl ether and diisopropyl ether. The esters can be, for example, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate and amyl acetate. The
15   ketones can be, for example, acetone, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone. Polar aprotic solvents can be, for example, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-methylpyrrolidone. Alkyl or cycloalkyl hydrocarbons can be, for example, cyclopentane, cyclohexane, cycloheptane, hexane, petroleum ether, heptane or mixtures thereof.

20   In another aspect, a process for preparation of crystalline rosuvastatin magnesium from rosuvastatin methyl ammonium salt of Formula II is provided.

The process comprises

   a)   lactonizing the rosuvastatin methyl ammonium salt of Formula II to give rosuvastatin lactone of Formula III;

25   b)   optionally isolating the rosuvastatin lactone of Formula III;

   c)   converting the rosuvastatin lactone to the rosuvastatin magnesium by treatment with a base and a magnesium salt; and

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d)   recovering the crystalline rosuvastatin magnesium from the reaction mass.

Rosuvastatin methyl ammonium salt of Formula II,
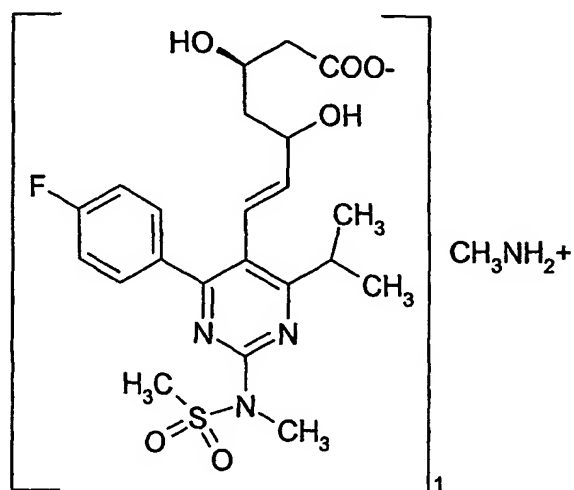
FORMULA II

5   can be prepared by the process described in PCT application WO 01/60804. Rosuvasatin
methyl ammonium salt can be treated with an acid (pH of about 1 to 5) to get rosuvastatin
lactone of Formula III

FORMULA III
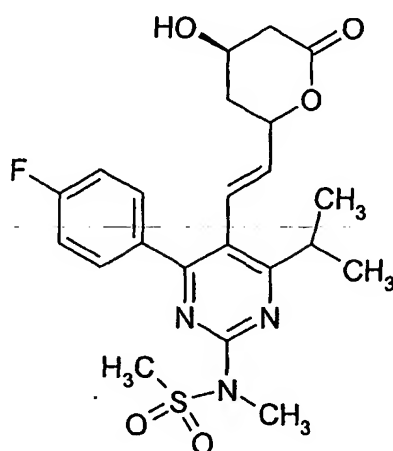
10   The reaction can be carried out in presence of a first organic solvent, optionally containing
water, at a temperature of about -10 to 100°C. After completion of the reaction, the layers
are separated and organic layer after washing with water and/or brine can be concentrated

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b)   removing solvent from the said solution; and

c)   recovering amorphous rosuvastatin magnesium.

Crystalline rosuvastatin magnesium can be prepared by methods described above.
The crystalline form can be dissolved in an organic solvent. The solvent can then be
5   removed from the solution to get amorphous rosuvastatin magnesium. The amorphous
rosuvastatin magnesium thus obtained can be dried using conventional drying techniques
such as vacuum tray drying, rotary vacuum drying, fluidized bed drier, tray drying and the
like.

The organic solvent can be, for example, lower alkanols, ethers, esters, ketones,
10   polar aprotic solvents or mixtures thereof. The lower alkanol can be, for example,
methanol, ethanol, isopropanol and n-propanol. The ethers can be, for example,
tetrahydrofuran and 1,4-dioxane. The esters can be, for example, ethyl formate, methyl
acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate
and amyl acetate. The ketones can be, for example, acetone, ethyl methyl ketone, methyl
15   isobutyl ketone and diisobutyl ketone. Polar aprotic solvents can be, for example, N,N-
dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-
methylpyrrolidone.

The solution of crystalline rosuvastatin magnesium can be prepared in the organic
solvent by optional warming to dissolve the solids. If required, the solution can be
20   clarified to remove the undissolved foreign particulate matter. The solution can then be
concentrated to remove solvent. The concentration can be carried out under vacuum of
about 100 to about 0.01 mm of Hg wherein the solvent is removed by vacuum distillation
while optionally heating the solution at a temperature of about 15 to about 55°C to effect
faster removal of the solvent.

25   The solvent can also be removed by spray-drying the solution of crystalline
rosuvastatin calcium using a spray-dryer. For the purpose of spray-drying, mini-spray
Dryer (Model: Buchi 190 Switzerland) which operates on the principle of nozzle spraying
in a parallel - flow i.e. the sprayed product and the drying gas flow in the same direction

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can be used, for example. The drying gas can be air or inert gases such as nitrogen, argon or carbon dioxide.

In another aspect, a process for preparing amorphous rosuvastatin magnesium from crystalline form is provided. The process comprises

5   a)   dissolving the crystalline form of rosuvastatin magnesium in a suitable first organic solvent;

       b)   adding a second organic solvent to the solution of rosuvastatin magnesium or adding the solution of rosuvastatin magnesium to the second organic solvent (in optional order of succession) in which rosuvastatin magnesium

10   is insoluble or very slightly soluble or sparingly soluble, such that amorphous rosuvastatin magnesium precipitates out from the solution; and

       c)   isolating the amorphous rosuvastatin magnesium from the mixture.

Crystalline rosuvastatin magnesium can be dissolved in a first organic solvent and to the solution added a second solvent or added solution of rosuvastatin magnesium to the

15 second organic solvent (in optional order of succession) in which rosuvastatin is insoluble or very slightly soluble or sparingly soluble. Due to addition of the second solvent, rosuvastatin magnesium precipitates out from the solution. The precipitated product can then be isolated and dried by conventional techniques to get the amorphous rosuvastatin magnesium.

20   The suitable first organic solvent can be for example lower alkanols, ethers, esters, ketones, polar aprotic solvents or mixtures thereof. The lower alkanol can be, for example, methanol, ethanol, isopropanol and n-propanol. The ethers can be, for example, tetrahydrofuran and 1,4-dioxane. The esters can be, for example, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate

25 and amyl acetate. The ketones can be, for example, acetone, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone. Polar aprotic solvents can be, for example, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-methylpyrrolidone.

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The second organic solvent in which rosuvastatin is insoluble (10,000 and over parts of solvent required for 1 part of solute as per United States Pharmacopoeia 2002) or very slightly soluble (form 1,000 to 10,000 parts of solvent required for 1 part of solute as per United States Pharmacopoeia 2002) or sparingly soluble (from 30 to 100 parts of

5   solvent required for 1 part of solute as per United States Pharmacopoeia 2002) can be, for example, isopropanol, isobutanol, n-butanol, cyclopentane, cyclohexane, cycloheptane, hexane, petroleum ether, heptane, diethyl ether, diisopropyl ether or mixtures thereof.

In another aspect, a process for preparation of amorphous rosuvastatin magnesium from crystalline rosuvastatin magnesiumis provided. The process comprises

10   a)   dissolving the crystalline rosuvastatin magnesium in a suitable organic
              solvent;

         b)   adding water to the solution of rosuvastatin magnesium, or adding the
              solution of rosuvastatin magnesium to water (in optional order of
              succession), such that rosuvastatin magnesium precipitates out from the
15   solution; and

         c)   isolating the amorphous rosuvastatin magnesium from the mixture.

Crystalline rosuvastatin magnesium can be dissolved in an organic solvent and the solution can be optionally treated with charcoal or clarified to remove foreign particulate matter. The clear solution can be obtained by gently warming the mixture as well. To the

20   clear solution water can be added at such a rate that rosuvastatin magnesium precipitates slowly. The mixture after complete addition of water can be chilled or partially concentrated to remove the organic solvent. The separated amorphous form can then be filtered and dried as per the methods described earlier.

The suitable organic solvent can be, for example, methanol, ethanol, isopropanol,

25   n-propanol, tetrahydrofuran, 1,4-dioxane, acetone, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-methylpyrrolidone or mixtures thereof.

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In another aspect, a process for preparing amorphous rosuvastatin magnesium from crystalline form is provided. The process comprises:,

a)   subjecting crystalline rosuvastatin magnesium to milling until the crystalline form is converted to amorphous form; and

5   b)   optionally drying the amorphous form.

Crystalline rosuvastatin magnesium solid or its slurry in an organic solvent can be milled by grinding between two surfaces. Such milling can be carried out by using a traditional technique of compounding using a pestle and mortar or by milling machines that essentially use the same principle. Examples of such milling machines include

10   various makes of ball mills, roller mills, gyratory mills, and the like. The slurry of crystalline rosuvastatin magnesium in an organic solvent can be of 30 to 85% w/v.

The organic solvent in which rosuvastatin is insoluble or very slightly soluble or sparingly soluble can be, for example, isopropanol, isobutanol, n-butanol, cyclopentane, cyclohexane, cycloheptane, hexane, petroleum ether, heptane, diethyl ether, diisopropyl

15   ether or mixtures thereof.

In another aspect, a process for preparing amorphous rosuvastatin magnesium from crystalline form is provided. The process comprises:

a)   dissolving crystalline rosuvastatin magnesium in an organic solvent optionally containing water; and

20   b)   freeze drying or lyophilizing the solution to get amorphous rosuvastatin magnesium.

A solution of crystalline rosuvastatin magnesium in an organic solvent optionally containing water can be prepared and treated with charcoal, filtered to remove charcoal. The clear solution is then freeze-dried by conventional techniques to get the amorphous

25   rosuvastatin magnesium. The amorphous form can then be dried under vacuum.

The organic solvent can be, for example, lower alkanols, ethers, esters, ketones, polar aprotic solvents or mixtures thereof. The lower alkanol can be, for example,

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methanol, ethanol, isopropanol and n-propanol. The ethers are selected from tetrahydrofuran and 1,4-dioxane. The esters can be, for example, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate and amyl acetate. The ketones can be, for example, acetone, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone. Polar aprotic solvents can be, for example, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-methylpyrrolidone.

In another aspect, a process for preparation of amorphous rosuvastatin magnesium from rosuvastatin methyl ammonium salt of Formula II is provided. The process comprises:

a) lactonizing the rosuvastatin methyl ammonium salt of Formula II;

b) optionally isolating the rosuvastatin lactone of Formula III;

c) converting the lactone form of rosuvastatin to rosuvastatin magnesium by treatment with a base and a magnesium salt;

d) removing the water from the reaction mass by azeotropic distillation using an organic solvent; and

e) recovering the amorphous form of rosuvastatin magnesium by removing the organic solvent from the resultant solution.

Rosuvastatin methyl ammonium salt can be converted to rosuvastatin magnesium as described above. However, after treating the aqueous layer with magnesium ions, to the reaction mass is added a suitable organic solvent capable or azeotropically removing water and simultaneously capable of dissolving rosuvastatin magnesium. From the resulting mixture, water was removed and from the solution of rosuvastatin magnesium, solvent was removed to get the desired amorphous rosuvastatin magnesium as solid.

Alternatively after treatment with magnesium ions, to the reaction mass is added an organic solvent which dissolves rosuvastatin magnesium and is immiscible or partially

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miscible with water. The solvent can be made immiscible in water by increasing the salinity of the aqueous layer, using for example, sodium chloride or calcium chloride. The layers can be separated and the organic layer containing rosuvastatin magnesium can then be dried, for example, over magnesium sulphate, sodium sulphate or molecular sieves to

5 remove traces of water. The organic layer can then be concentrated to remove solvent, and get the desired amorphous rosuvastatin magnesium. The concentration can be effected by either spray drying or by vacuum distillation.

The organic solvent can be, for example, tetrahydrofuran, 1,4-dioxane, toluene, xylene, dichloromethane, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-

10 propyl acetate, isobutyl acetate, butyl acetate, amyl acetate, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone or mixtures thereof.

In another aspect, a process of preparation of amorphous rosuvastatin magnesium from crystalline form is provided. The process comprises:

a)   treating crystalline rosuvastatin magnesium with an acid to obtain
15   rosuvastatin;

b)   optionally isolating rosuvastatin;

c)   converting rosuvastatin to rosuvastatin magnesium by treatment with a
base and magnesium salt;

d)   removing water from the reaction mass by azeotropic distillation using an
20   organic solvent; and

e)   recovering the amorphous form of rosuvastatin magnesium by removing
the organic solvent from the resultant solution.

Crystalline rosuvastatin magnesium can be treated with an acid to get rosuvastatin acid. The conversion can be easily carried out in presence of water optionally containing

25 an organic solvent. The reaction temperature can be kept at about -5 to 100°C for example.

The organic solvent can be, for example, lower alkanols, ethers, esters, ketones, polar aprotic solvents, alkyl or cycloalkyl hydrocarbons or mixtures thereof. The lower

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alkanol can be, for example, methanol, ethanol, isopropanol, isobutanol, n-butanol and n-propanol. The ethers can be, for example, tetrahydrofuran, 1,4-dioxane, diethyl ether and diisopropyl ether. The esters can be, for example, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate and amyl

5   acetate. The ketones can be, for example, acetone, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone. Polar aprotic solvents can be, for example, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide, acetonitrile and N-methylpyrrolidone. Alkyl or cycloalkyl hydrocarbons can be, for example, cyclopentane, cyclohexane, cycloheptane, hexane, petroleum ether, heptane or mixtures thereof.

10   .   After completion of the reaction the aqueous layer can be extracted with organic solvent, as described above. The organic layer after evaporation of solvent under vacuum gives rosuvastatin acid as oily residue. The residue can be dissolved in water and organic solvent and treated with a base and magnesium ions to get rosuvastatin magnesium. To the reaction mass can be added a suitable second organic solvent capable of azeotropically

15   removing water and simultaneously capable of dissolving rosuvastatin magnesium. From the resulting mixture water was removed and from the solution of rosuvastatin magnesium, solvent was removed to get the desired amorphous rosuvastatin magnesium as solid.

Alternatively after treatment with magnesium ions, to the reaction mass can be

20   added a second organic solvent which dissolves rosuvastatin magnesium and is immiscible or partially miscible with water. The solvent can be made immiscible in water by increasing the salinity of the aqueous layer using, for example, sodium chloride or calcium chloride. The layers are separated and the organic layer containing rosuvastatin magnesium is then dried over magnesium sulphate, sodium sulphate or molecular sieves to

25   remove traces of water. The organic layer can then be concentrated to remove solvent to get the desired amorphous rosuvastatin magnesium. The concentration can be effected by either spray-drying or by vacuum distillation.

The second organic solvent can be, for example, tetrahydrofuran, 1,4-dioxane, toluene, xylene, dichloromethane, ethyl formate, methyl acetate, ethyl acetate, isopropyl

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acetate, n-propyl acetate, isobutyl acetate, butyl acetate, amyl acetate, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone or mixtures thereof.

The examples of base and magnesium salt are described in above.

The acid used can be, for example, inorganic acid such as hydrochloric acid, sulphuric acid, phosphoric acid, hydrobromic acid, nitric acid and the like or a mixture thereof; or an organic acid, such as formic acid, acetic acid, propionic acid, anhydrides of carboxylic acids, methanesulphonic acid, 4-toluenesulphonic acid and the like.

In another aspect, a process for preparation of amorphous rosuvastatin magnesium from rosuvastatin methyl ammonium salt of Formula II is provided. The process comprises:,

a)   treating the methyl ammonium salt form of rosuvastatin with a base and a calcium salt;

b)   removing the water from the reaction mass by azeotropic distillation using an organic solvent; and

c)   recovering the amorphous form of rosuvastatin calcium by removing the organic solvent from the resultant solution.

Rosuvastatin methyl ammonium salt can be converted to rosuvastatin magnesium as described above. However, after treating the aqueous layer with magnesium ions, to the reaction mass can be added a suitable organic solvent capable of azeotropically removing water and simultaneously capable of dissolving rosuvastatin magnesium. From the resulting mixture water can be removed and from the solution of rosuvastatin calcium, solvent can be removed to get the desired amorphous rosuvastatin magnesium as solid.

Alternatively after treatment with magnesium ions, to the reaction mass can be added an organic solvent which dissolves rosuvastatin magnesium and is immiscible or partially miscible with water. The solvent can be made immiscible in water by increasing the salinity of the aqueous layer using, for example, sodium chloride or calcium chloride.

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The layers are separated and the organic layer containing rosuvastatin calcium is then dried over calcium chloride, sodium sulphate or molecular sieves to remove traces of water. The organic layer can then be concentrated to remove solvent to get the desired amorphous rosuvastatin magnesium. The concentration can be effected by either spray-drying or by vacuum distillation.

The organic solvent can be, for example, tetrahydrofuran, 1,4-dioxane, toluene, xylene, dichloromethane, ethyl formate, methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, butyl acetate, amyl acetate, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone or mixtures thereof.

In another aspect, a process for converting a mixture of amorphous and crystalline form of rosuvastatin magnesium to completely amorphous rosuvastatin magnesium is provided.

A mixture of largely amorphous with some crystalline rosuvastatin magnesium can be prepared directly from the rosuvastatin lactone or rosuvastatin methyl ammonium salt or from the crystalline form by the process already described in the specification with little variations. The crystalline rosuvastatin magnesium can be converted to a mixture of amorphous and crystalline material by techniques described above.

The mixture of largely amorphous rosuvastatin magnesium containing some crystalline form can then be converted to the amorphous form by techniques described Above.

In another aspect, the use of amorphous rosuvastatin magnesium in the preparation of rosuvastatin calcium either in crystalline or in amorphous form is provided. The aspect further provides a process for preparation of rosuvastatin calcium from amorphous rosuvastatin magnesium. The process comprises

a)   reating amorphous rosuvastatin magnesium with a base and a calcium salt; and

b)   isolating rosuvastatin calcium from the reaction mass.

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The amorphous rosuvastatin magnesium can be treated with a base in presence of water optionally containing an organic solvent. The resultant mass can be concentrated partially to remove the organic solvent, followed by addition of the solution of calcium salt in water under vigorous stirring at such a rate that amorphous rosuvastatin calcium
5   separates out from the reaction mass slowly. After complete addition the mass can be further stirred at temperature of about 10 to 30°C for about 1 to about 10 hours and filtered to get amorphous rosuvastatin calcium, which can be dried, for example, under vacuum.

The amorphous rosuvastatin calcium can then be crystallized by methods described in, for example, US Patent No. 6,589,959 to get crystalline rosuvastatin calcium which can
10   be further converted to amorphous rosuvastatin calcium by methods described in, for example, co-pending Indian application 1304/DEL/2003.

In another aspect, amorphous rosuvastatin magnesium having an X-ray diffraction pattern as depicted in Figure I is provided.

In another aspect, pharmaceutical compositions and dosage forms comprising the
15   amorphous rosuvastatin magnesium to be used as HMG-CoA reductase inhibitor in treatment of hyperlipidemia are provided.

In another aspect, a method inhibiting HMG-CoA enzyme in treatment of hyperlipidemia, comprising administering to a mammal in need thereof a therapeutically effective amount of the amorphous rosuvastatin magnesium is provided.

20   While the present invention has been described in terms of its specific embodiments, certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.

Example 1: Preparation of Crystalline Rosuvastatin Magnesium from Rosuvastatin Methyl Ammonium Salt

25   Rosuvastatin methyl ammonium salt of Formula II (10 g) was added into water (50 ml) and to the resultant mass added a solution of aqueous sodium hydroxide (9 ml, 8% w/v) at 25-30oC and stirred for further 30 min. The solution was filtered through celite bed and the bed was washed with water (20 ml). The reaction mass concentrated to

recover water (40 ml) under vacuum at 60°C. To the reaction mass was added water (40 ml) and the equivalent quantity of water was removed under vacuum. The final solution was diluted with water (50 ml). To the solution after dilution with water was added aqueous solution of magnesium diacetate tetrahydrate (2.5 g, 20%) at 35°C with vigorous agitation. The resultant mass was further stirred for 1 hour at room temperature and solid crystalline product was collected by filtration. The product was dried under vacuum at 50°C.

Yield: 7.8 g (80%) (XRD as per Figure 2 showed it to be crystalline material)

HPLC Purity: 99.51%

Diastereomeric impurity: 0.27%

Example 2: Preparation of Crystalline Rosuvastatin Magnesium from Rosuvastatin Lactone

Rosuvastatin lactone of Formula III (5.0 g) was suspended in methanol (50 ml) and water (25 ml) at 25-30°C. To this mixture was added solution of aqueous sodium hydroxide (4.5 ml, 8% w/v) at 25-30°C and then further stirred at 35°C for 1 hour. After complete hydrolysis of lactone, methanol was recovered under reduced pressure at temperature below 45°C. To this solution was added water (15 ml) and a solution of magnesium diacetate tetrahydrate (1.34 g) in water (5 ml) at 35°C under vigorous agitation. The reaction mass was further stirred for 1 hour at 35°C and then cooled to 25-30°C. After complete precipitation of magnesium salt, it was filtered and the cake was washed with water (15 ml). The product was dried at 45 to 50°C.

Yield: 4.8 g (85%) (XRD as per Figure 2 showed it to be crystalline material)

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Example 3: Preparation Of Crystalline Rosuvastatin Magnesium From Rosuvastatin Methyl Ammonium Salt

Step A) Preparation of Rosuvastatin Lactone from Rosuvastatin Methyl Ammonium Salt.

5   Rosuvastatin methyl ammonium salt (20 gm) was added into mixture of ethyl acetate (100 ml) and water (200 ml) at 25-30°C and the pH of the reaction mass was adjusted to about 3.0 with 6N hydrochloric acid. The layers were separated and the organic layer is washed with water (50 ml). The organic layer was concentrated under vacuum to get an oily crude product, which was mixed with toluene (50 ml). The reaction 10 mass was refluxed for about 6 hours and the solvent was removed under vacuum at 60°C. The residue obtained was stirred with hexane (100 ml) and the separated solid was filtered. Dried the product under vacuum till constant weight at 40-45°C to get rosuvastatin lactone.

Step B) Preparation of Crystalline Rosuvastatin Magnesium from Rosuvastatin 15 Lactone

   Rosuvastatin lactone of Formula III (5.0 g) was suspended in methanol (50 ml) and water (25 ml) at 25-30°C. To this mixture was added solution of aqueous sodium hydroxide (4.5 ml, 8% w/v) at 25-30°C and then further stirred at 35°C for 1 hour. After complete hydrolysis of lactone, methanol was recovered under reduced pressure at 20 temperature below 45°C. To this solution was added water (15 ml) and a solution of magnesium diacetate tetrahydrate (1.34 g) in water (5 ml) at 35°C under vigorous agitation. The reaction mass was further stirred for 1 hour at 35°C and then cooled to 25-30°C. After complete precipitation of magnesium salt, it was filtered and the cake was washed with water (15 ml). The product was dried at 45 to 50°C.

25 Yield: 4.8 g (85%) (XRD as per Figure 2 showed it to be crystalline material)

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Example 4: Preparation of Amorphous Rosuvastatin Magnesium from Crystalline Rosuvastatin Magnesium By Vacuum Drying

Crystalline Rosuvastatin magnesium (2.0 g) was dissolved in tetrahydrofuran (5 ml) at 25-30°C. The solution was filtered through celite bed to get a clear solution of
5   rosuvastatin magnesium in tetrahydrofuran and the celite bed was washed with tetrahydrofuran (2 ml). The clear solution was then concentrated and solvent was recovered under vacuum at 45°C to get the title product.

Yield: 1.9 g (95%) (XRD as per Figure 1 showed it to be amorphous material)

HPLC Purity: 99.41%

10   Diastereomeric impurity: 0.34%

Example 5: Preparation of Amorphous Rosuvastatin Magnesium from Crystalline Rosuvastatin Magnesium By Spray Drying

Crystalline Rosuvastatin magnesium (5.0 g) was dissolved in tetrahydrofuran (25 ml) at 25-30°C. The solution was filtered through celite bed to get a clear solution of
15   rosuvastatin magnesium in tetrahydrofuran and the celite bed was washed with tetrahydrofuran (2 ml). The clear solution obtained was fed in to spray drier at 25-30°C at nitrogen flow rate of 600 Newtonlitre per hr and product was collected and dried under vacuum at 45°C.

Yield: 4.5 g (90%) (XRD as per Figure 1 showed it to be amorphous material)

20   Example 6: Preparation of Amorphous Rosuvastatin Magnesium from Crystalline Rosuvastatin Magnesium By Precipitation

Crystalline Rosuvastatin magnesium (2.0 g) was dissolved in tetrahydrofuran (5 ml) at 25-30°C. The solution was filtered through celite bed to get a clear solution of rosuvastatin magnesium in tetrahydrofuran and the celite bed was washed with
25   tetrahydrofuran (2 ml).   The resultant solution was poured dropwise into cyclohexane (60 ml) under vigorous agitation after which the mixture was further stirred for 30 min.

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The solid product which precipitated was filtered at 25-30°C under nitrogen and dried under vacuum at 45°C.

Yield: 1.7 g (85%) (XRD as per Figure 1 showed it to be amorphous material)

HPLC Purity: 99.27% Diastereomeric impurity: 0.41%

5   Example 7: Preparation of Amorphous Rosuvastatin Magnesium from Crystalline Rosuvastatin Magnesium By Milling

Crystalline rosuvastatin magnesium (2.0 gm) was slurried in cyclohexane (10 ml) and the slurry was placed in a glass mortar. The slurry was triturated with pestle till the crystalline form was completely converted to amorphous form. The slurry was then

10   filtered and the solid was dried under vacuum at 40-45°C to get amorphous rosuvastatin magnesium.

Yield: 1.3 gm (65%) (XRD as per Figure 1 showed it to be an amorphous material)

Example 8: Preparation of Rosuvastatin Magnesium Amorphous grom Crystalline Rosuvastatin Magnesium By Freeze Drying

15   Crystalline rosuvastatin magnesium (1.0 g) was dissolved in 1,4 dioxane (5 ml) by gently warming at 38°C. The solution was placed in a bath containing acetone and dry ice maintained at about -20 to -30°C and cooled to –20°C under rotation. The material was solidified by cooling and then vacuum of less than 0.1 mm of Hg was applied. The temperature of the bath was slowly raised from –20 to 10°C over 2 hours while under

20   vacuum. Further temperature was raised to 10 to 25°C over 1 hour. Title compound was obtained was dried under vacuum at 45°C.

Yield: 0.85 g (85%) (XRD as per Figure 1 showed it to be amorphous material)

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Example 9: Preparation of Amorphous Rosuvastatin Magnesium from Rosuvastatin
Methyl Ammonium Salt

**Step A) Preparation of Rosuvastatin Lactone from Rosuvastatin Methyl Ammonium
Salt.**

5   Rosuvastatin methyl ammonium salt (20 gm) was added into a mixture of ethyl
acetate (100 ml) and water (200 ml) at 25-30°C and the pH of the reaction mass was
adjusted to about 3.0 with 6N hydrochloric acid. The layers were separated and the
organic layer washed with water (50 ml). The organic layer was concentrated under
vacuum to get an crude oily product, which was mixed with toluene (50 ml). The reaction
10   mass was refluxed for about 6 hours and the solvent was removed under vacuum at 60°C.
The residue obtained was stirred with hexane (100 ml) and the separated solid was filtered.
Dried the product under vacuum to constant weight at 40-45°C to get rosuvastatin lactone.

**Step B) Preparation of Amorphous Rosuvastatin Magnesium from Rosuvastatin
Lactone**

15   Rosuvastatin lactone of Formula III (5.0 g) was suspended in methanol (50 ml) and
water (25 ml) at 25-30°C.   To this mixture was added solution of aqueous sodium
hydroxide (4.5 ml, 8% w/v) at 25-30°C and then further stirred at 35°C for 1 hour. After
complete hydrolysis of lactone, methanol was recovered under reduced pressure at
temperature below 45°C. To this solution was added water (15 ml) and aqueous solution
20   of Magnesium diacetate tetrahydrate (1.34 g, 26% w/v) was added into reaction mixture at
35°C under vigorous agitation. Solid compound was precipitated out. Added to the slurry
tetrahydrofuran (70 ml) and stirred for 10 minutes. Solid sodium chloride (2 g) was added
to the reaction mass and further stirred for 25 minutes after which the layers were
separated. The organic layer was dried over powdered molecular sieves (10 g) for
25   sufficient period followed by filtration to remove the molecular sieves. The clear filtrate
was concentrated under vacuum to remove remaining water azeotropically with
tetrahydrofuran. The crude product obtained after removal of tetrahydrofuran completely
was dissolved into fresh tetrahydrofuran (50 ml) and filtered to remove any undissolved
particles. The organic layer was concentrated under vacuum at 45°C to remove the solvent
30   completely to get title compound dried under vacuum at 45°C.

Yield: 3.5 g (70%) (XRD as per Figure 1 showed it to be amorphous material)

Example 10: Conversion of Crystalline Rosuvastatin Magnesium to Amorphous
Rosuvastatin Magnesium

Crystalline rosuvastatin magnesium (10.0 g) was added into mixture of ethyl
acetate (100 ml) and water (100 ml) at room temperature. The pH of the resulting solution
was adjusted 4.0 to 4.2 by adding dilute hydrochloric acid at 25°C. The layers were
separated and organic layer was washed with water. The solvent was concentrated under
vacuum to get oily residue.

The oily residue obtained above was dissolved in methanol (35 ml) and water (50
ml) at room temperature. The pH of the solution was adjusted with sodium hydroxide (8%
solution in water) to about 8.5 to 9.0 and the resulting reaction mass was stirred for further
1 hour at room temperature. Methanol was removed under vacuum. The oily residue was
reconstituted in water (50 ml) and to the aqueous solution added a solution of magnesium
diacetate tetrahydrate (2.8 g) in water (20 ml) at 20-22°C with vigorous stirring. Solid
compound was precipitated out. Added to the slurry tetrahydrofuran (50 ml) and stirred
for 10 minutes. Solid sodium chloride (2 g) was added to the reaction mass and further
stirred for 25 minutes after which the layers were separated. The organic layer was dried
over powdered molecular sieves (10 g) for sufficient period followed by filtration to
remove the molecular sieves. The clear filtrate was concentrated under vacuum to remove
remaining water azeotropically with tetrahydrofuran. The crude product obtained after
removal of tetrahydrofuran completely was dissolved into fresh tetrahydrofuran (50 ml)
and filtered to remove any undissolved particles. The organic layer was concentrated
under vacuum at 45°C to remove the solvent completely to get title compound dried under
vacuum at 45°C.

Yield: 7.8 g (78%) (XRD as per Figure 1 showed it to be amorphous material)

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Example 11: Preparation of Amorphous Rosuvastatin Magnesium from Rosuvastatin
Methyl Ammonium Salt

Rosuvastatin methyl ammonium salt of Formula II (10.0 g) was added into water
(50 ml) and to it charged aqueous sodium hydroxide solution (9 ml, 8% w/v) at 25-30°C.
5   The resultant mass was stirred for 20 minutes. The mass was filtered through celite bed
and the bed was washed with water (20 ml). From the resultant clear solution water (40
ml) was recovered under vacuum at 60°C followed by addition of further water (40 ml).
The mass was further concentrated to recover water (40 ml). After removal of water was
added fresh water (40 ml) to the reaction mass. Aqueous solution of Magnesium diacetate
10 tetrahydrate (2.6 g, 26% w/v) was added into reaction mixture at 35°C under vigorous
agitation. Solid compound was precipitated out. Added to the slurry tetrahydrofuran (50
ml) and stirred for 10 minutes. Solid sodium chloride (2 g) was added to the reaction mass
and further stirred for 25 minutes after which the layers were separated. The organic layer
was dried over powdered molecular sieves (10 g) for sufficient period followed by
15 filtration to remove the molecular sieves. The clear filtrate was concentrated under
vacuum to remove remaining water azeotropically with tetrahydrofuran. The crude
product obtained after removal of tetrahydrofuran completely was dissolved into fresh
tetrahydrofuran (50 ml) and filtered to remove any undissolved particles. The organic
layer was concentrated under vacuum at 45°C to remove the solvent completely to get title
20 compound dried under vacuum at 45°C.

Yield: 6.5 g (68%) (XRD as per Figure 1 showed it to be amorphous material)

Example 12: Preparation of Amorphous Rosuvastatin Magnesium from Crystalline
Rosuvastatin Magnesium

Step A) Preparation of Mixture of Amorphous and Crystalline Rosuvastatin
25 **Magnesium**

Crystalline rosuvastatin magnesium (6.0 gm) was slurried in cyclohexane (10 ml)
and the slurry was placed in a glass mortar. The slurry was triturated with pestle for about
25 to 30 hours. The slurry was then filtered and the solid was dried under vacuum at 40-
45°C to get the tile compound.

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Yield: 5.43 gm (90%) (XRD as per Figure 3 showed it to be a mixture of amorphous and crystalline material)

Step B) Conversion of Mixture of Amorphous and Crystalline Rosuvastatin Magnesium to Amorphous Rosuvastatin Magnesium

5   The product obtained in step A) (2.0 g) was dissolved in tetrahydrofuran (5 ml) at 25-30°C. The solution was filtered through celite bed to get a clear solution of rosuvastatin magnesium in tetrahydrofuran and the celite bed was washed with tetrahydrofuran (2 ml). The clear solution was then concentrated and solvent was recovered under vacuum at 45°C to get title product.

10   Yield: 1.9 g (95%) (XRD as per Figure 1 showed it to be amorphous material)

Example 13: Preparation of Amorphous Rosuvastatin Calcium from Amorphous Rosuvastatin Magnesium

       Amorphous rosuvastatin magnesium (10.0 g) was added into mixture of ethyl acetate (100 ml) and water (100 ml) at room temperature. The pH of the resulting solution 15   was adjusted 4.0 to 4.2 by adding dilute hydrochloric acid at 25°C. The layers were separated and organic layer was washed with water. The solvent was concentrated under vacuum to get oily residue.

       The oily residue obtained above was dissolved in methanol (35 ml) and water (50 ml) at room temperature. The pH of the solution was adjusted with sodium hydroxide (8% 20   w/v solution in water) to about 8.5 to 9.0 and the resulting reaction mass was stirred for further 1 hour at room temperature. Methanol was removed under vacuum. The oily residue was reconstituted in water (50 ml) and to the aqueous solution added a solution of calcium acetate (2.1 gm) in water (10 ml) at 20-22oC with vigorous stirring. After complete addition, mixture was stirred for further 2 hours at 20-22oC and filtered, washed 25   the cake with water (20 ml) thrice and then dried at 45oC under vacuum to get amorphous rosuvastatin calcium.

Yield: 7.50 gm (75%) (XRD as per Figure 4 showed it to be an amorphous material)

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HPLC Purity: 99.52% Diastereomeric impurity: 0.27%

Example 14: Preparation of Crystalline Rosuvastatin Calcium from Amorphous
Rosuvastatin Calcium

The product obtained in Example 13 (5.0 gm) was added to a mixture of water (50
ml) and acetonitrile (50 ml) at 15oC. The mixture was warmed to 40oC to obtain complete
solution. The mixture was then cooled slowly to 25-30oC and stirred for 16 hours. The
crystalline product was separated by filtration at ambient temperature and dried at 50oC
under vacuum to give rosuvastatin calcium as white crystals.

Yield: 3.4 gm (68%) (XRD as per Figure 5 showed it to be crystalline material)

We Claim:

1. Amorphous rosuvastatin magnesium.

2. Amorphous rosuvastatin magnesium having purity greater than 99% with diastereomeric impurity less than 0.5%.

3. Amorphous rosuvastatin magnesium according to claim 2 having purity greater than 99.5% with diastereomeric impurity less than 0.25%.

4. Amorphous rosuvastatin magnesium according to claim 3 having purity greater than 99.8% with diastereomeric impurity less than 0.15%.

5. Amorphous rosuvastatin magnesium substantially free of crystalline rosuvastatin magnesium.

6. A process for the preparation of crystalline rosuvastatin magnesium comprising:

   a) treating rosuvastatin methyl ammonium salt or rosuvastatin lactone with a base and magnesium salt; and

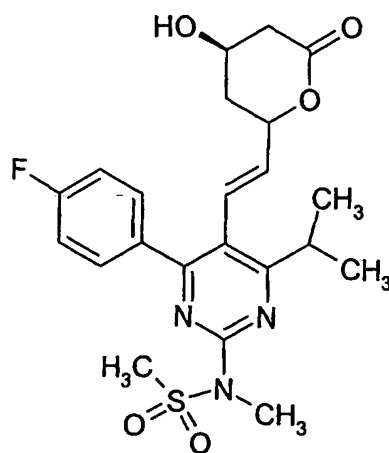   b) isolating crystalline rosuvastatin magnesium from the reaction mass.

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7.   A process for preparation of crystalline rosuvastatin magnesium from rosuvastatin methyl ammonium salt of Formula II,
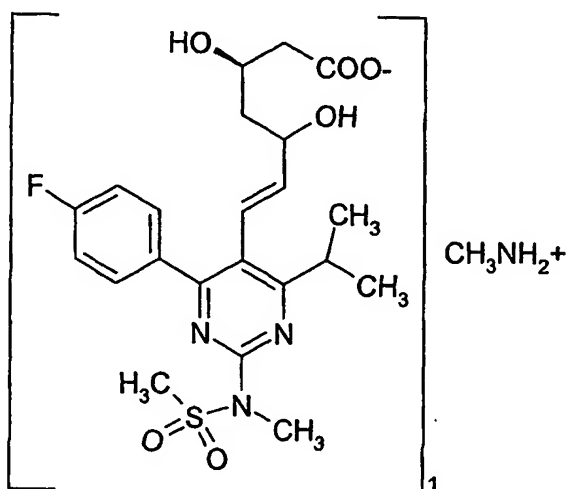
FORMULA II

Comprising;

a)   lactonizing the rosuvastatin methyl ammonium salt of Formula II;

b)   optionally isolating the rosuvastatin lactone of Formula III;
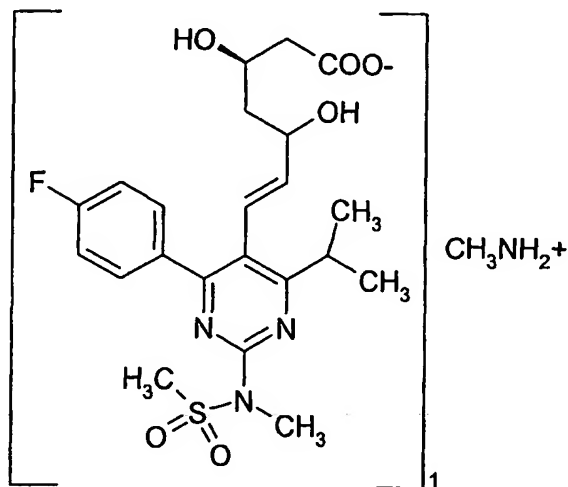

FORMULA III

- 30 -

c)   converting the rosuvastatin lactone to the rosuvastatin magnesium by treatment with a base and a magnesium salt; and

d)   recovering the crystalline rosuvastatin magnesium from the reaction mass.

8.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   dissolving crystalline rosuvastatin magnesium in an organic solvent;

b)   removing the solvent from the solution; and

c)   recovering amorphous rosuvastatin magnesium.

9.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   dissolving crystalline rosuvastatin magnesium in a first organic solvent;

b)   adding a second organic solvent to the solution of rosuvastatin magnesium or adding the solution of rosuvastatin magnesium to the second organic solvent (in optional order of succession) wherein rosuvastatin magnesium is insoluble or very slightly soluble or sparingly soluble in the second solvent, such that amorphous rosuvastatin magnesium precipitates; and

c)   isolating amorphous rosuvastatin magnesium.

10.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   dissolving crystalline rosuvastatin magnesium in an organic solvent;

b)   adding water to the solution of rosuvastatin magnesium, or adding the solution of rosuvastatin magnesium to water (in optional order of succession), such that rosuvastatin magnesium precipitates; and

c)   isolating amorphous rosuvastatin magnesium.

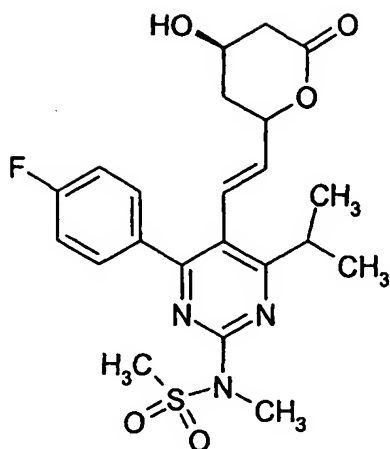11.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   subjecting crystalline rosuvastatin magnesium to milling until the crystalline form is converted to the amorphous form; and

b)   optionally drying the amorphous form.

12.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   dissolving crystalline rosuvastatin magnesium in an organic solvent optionally containing water; and

b)   freeze drying or lyophilizing the solution to get amorphous rosuvastatin magnesium.

13.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   lactonizing rosuvastatin methyl ammonium salt of Formula II; and

FORMULA II

b)   optionally isolating the rosuvastatin lactone of Formula III;

FORMULA III

c)   converting the lactone form of rosuvastatin to the rosuvastatin magnesium
by treatment with a base and a magnesium salt;

d)   removing the water from the reaction mass by azeotropic distillation using
an organic solvent; and

e)   recovering the amorphous form of rosuvastatin magnesium by removing
the organic solvent from the resultant solution.

14.   A process for preparing amorphous rosuvastatin magnesium comprising:

a)   treating crystalline rosuvastatin magnesium with an acid to obtain
rosuvastatin;

b)   optionally isolating rosuvastatin;

c)   converting rosuvastatin to rosuvastatin magnesium by treatment with a base
and magnesium salt;

d)   removing the water from the reaction mass by azeotropic distillation using
an organic solvent; and

e)   recovering amorphous rosuvastatin magnesium by removing the organic
solvent from the resultant solution.

15.   A process for preparation of amorphous rosuvastatin magnesium comprising:

    a)   treating rosuvastatin methyl ammonium salt with a base and a magnesium
          salt;

    b)   removing water from the reaction mass by azeotropic distillation using an
          organic solvent; and

    c)   recovering amorphous rosuvastatin magnesium by removing the organic
          solvent from the resultant solution.

16.   A process for preparing rosuvastatin calcium comprising:

    a)   treating amorphous rosuvastatin magnesium with a base and a calcium salt;
          and

    b)   isolating rosuvastatin calcium from the reaction mass.

17.   Amorphous rosuvastatin magnesium having an X-ray diffraction pattern as
       depicted in Figure 1.

18.   Pharmaceutical composition to be used as HMG-CoA reductase inhibitor in
       treatment of hyperlipidemia comprising amorphous rosuvastatin magnesium.

19.   A method for inhibiting HMG-CoA enzyme in treatment of hyperlipidemia,
       comprising administering to a mammal in need thereof a therapeutically effective
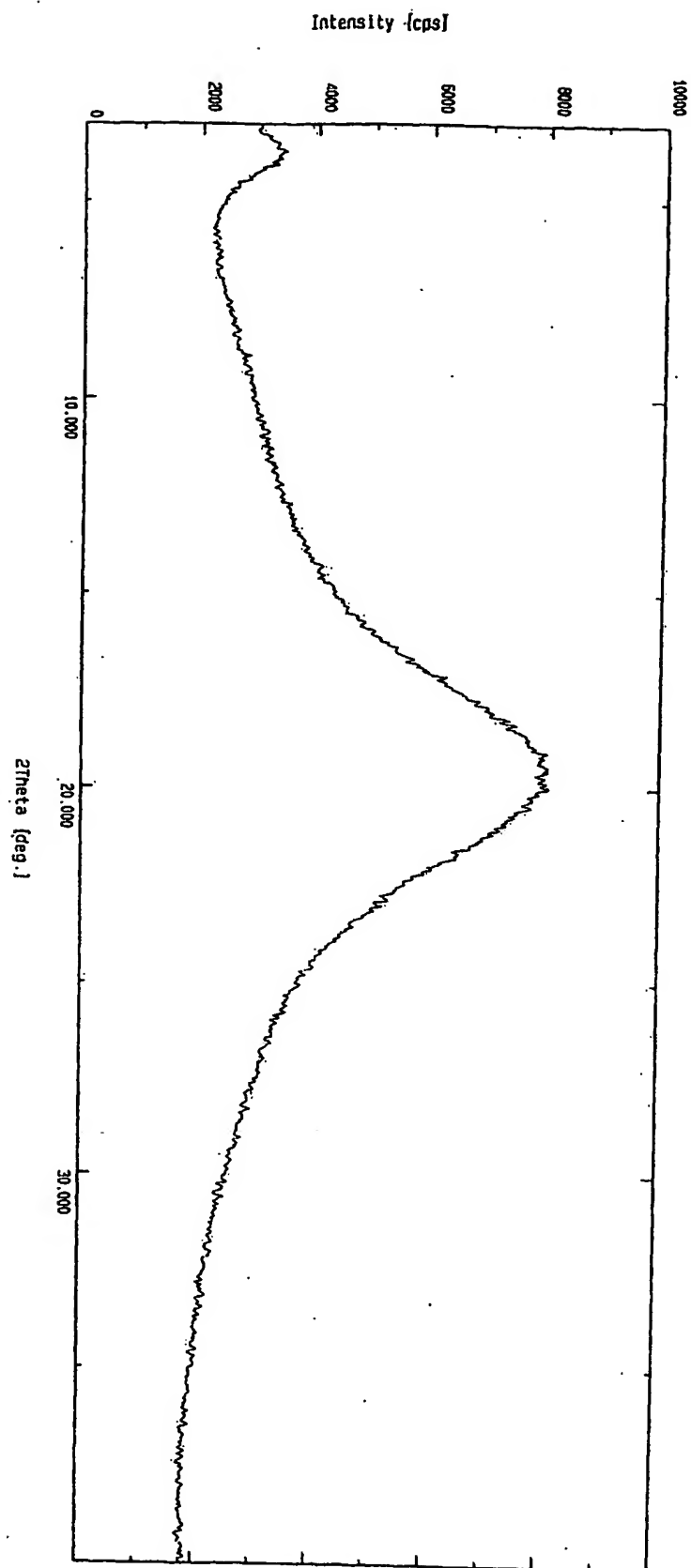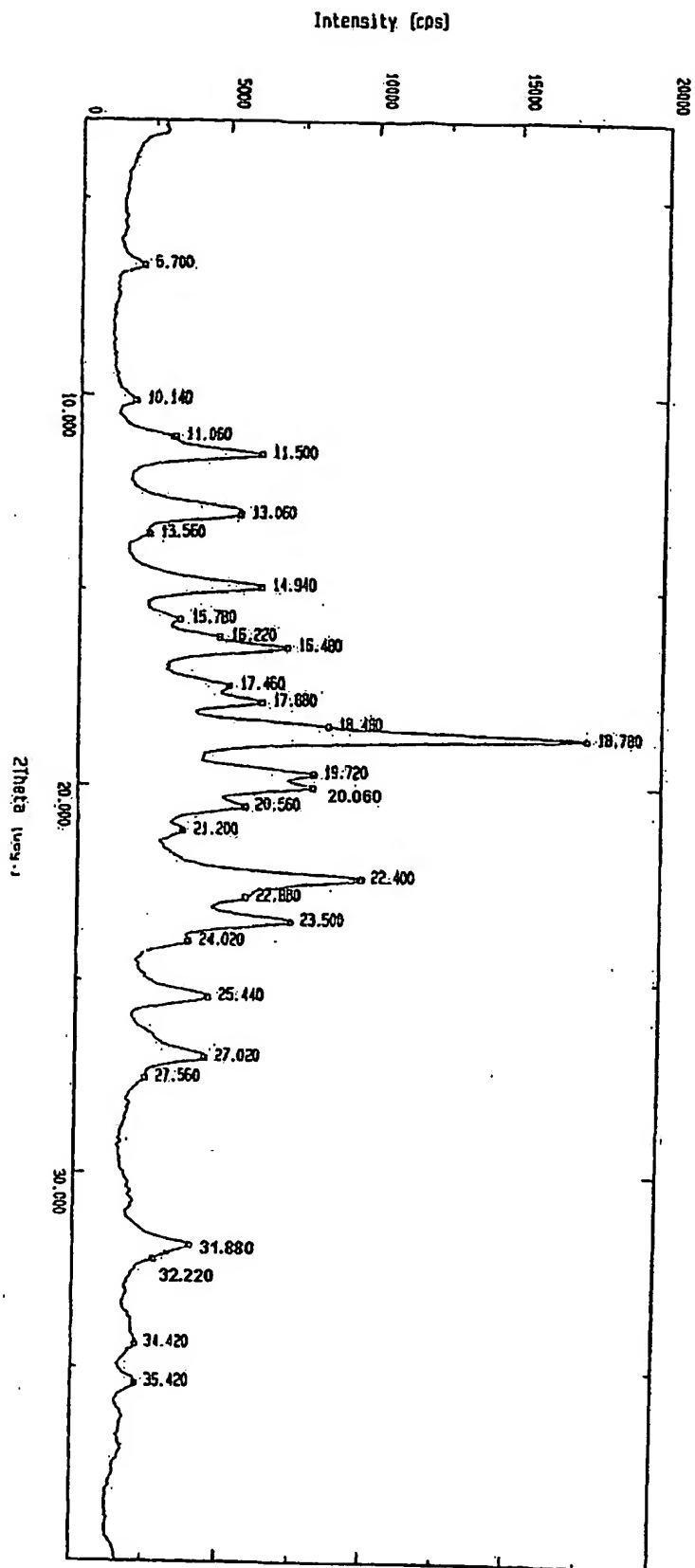   amount of amorphous rosuvastatin magnesium.
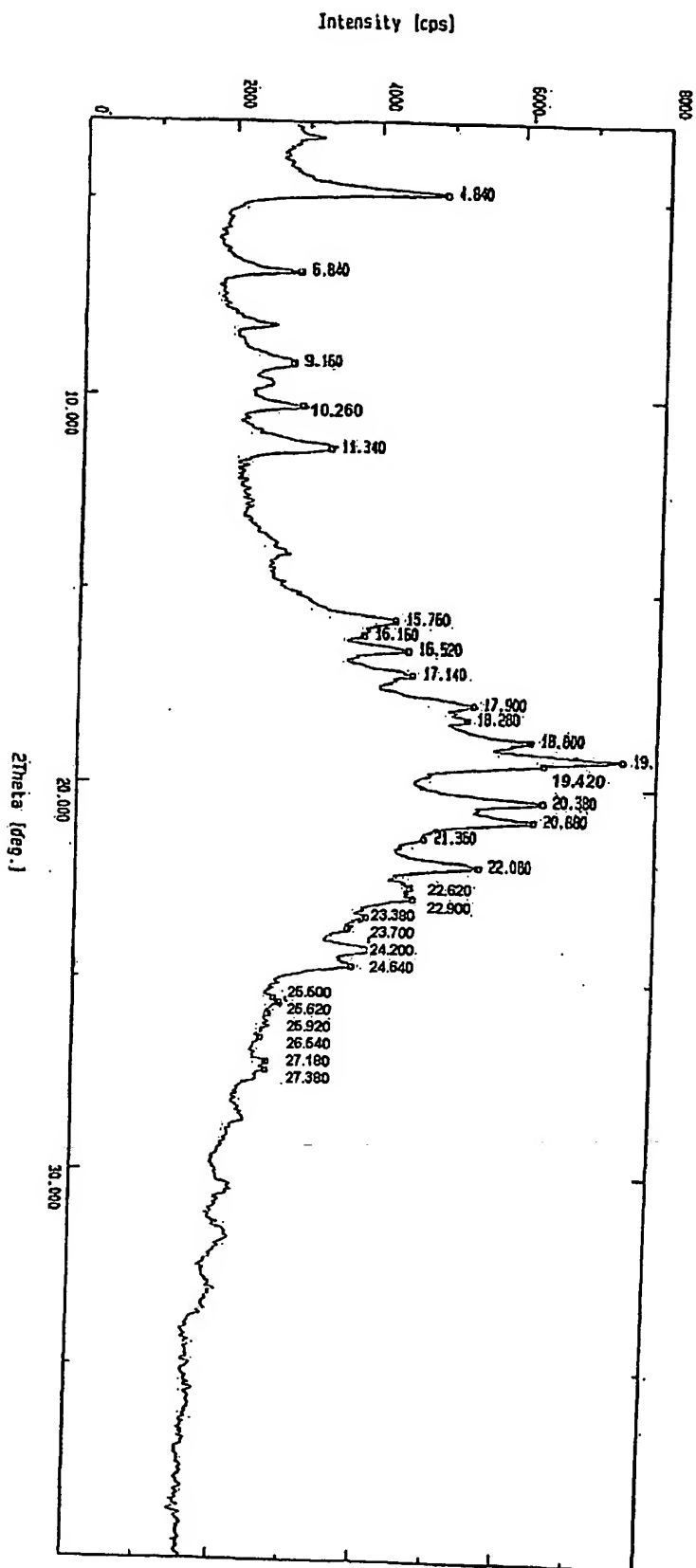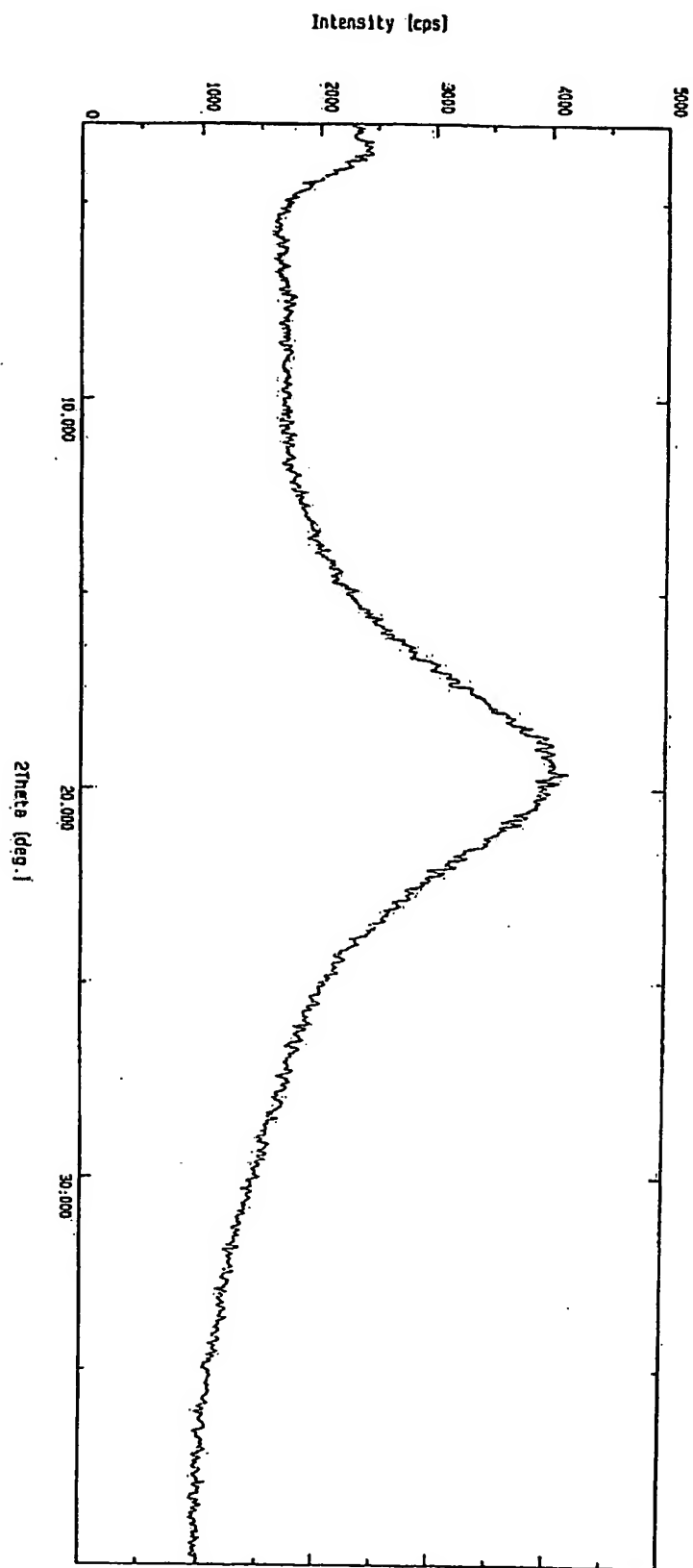
Figure 1

Figure 2

Figure 3

Figure.4
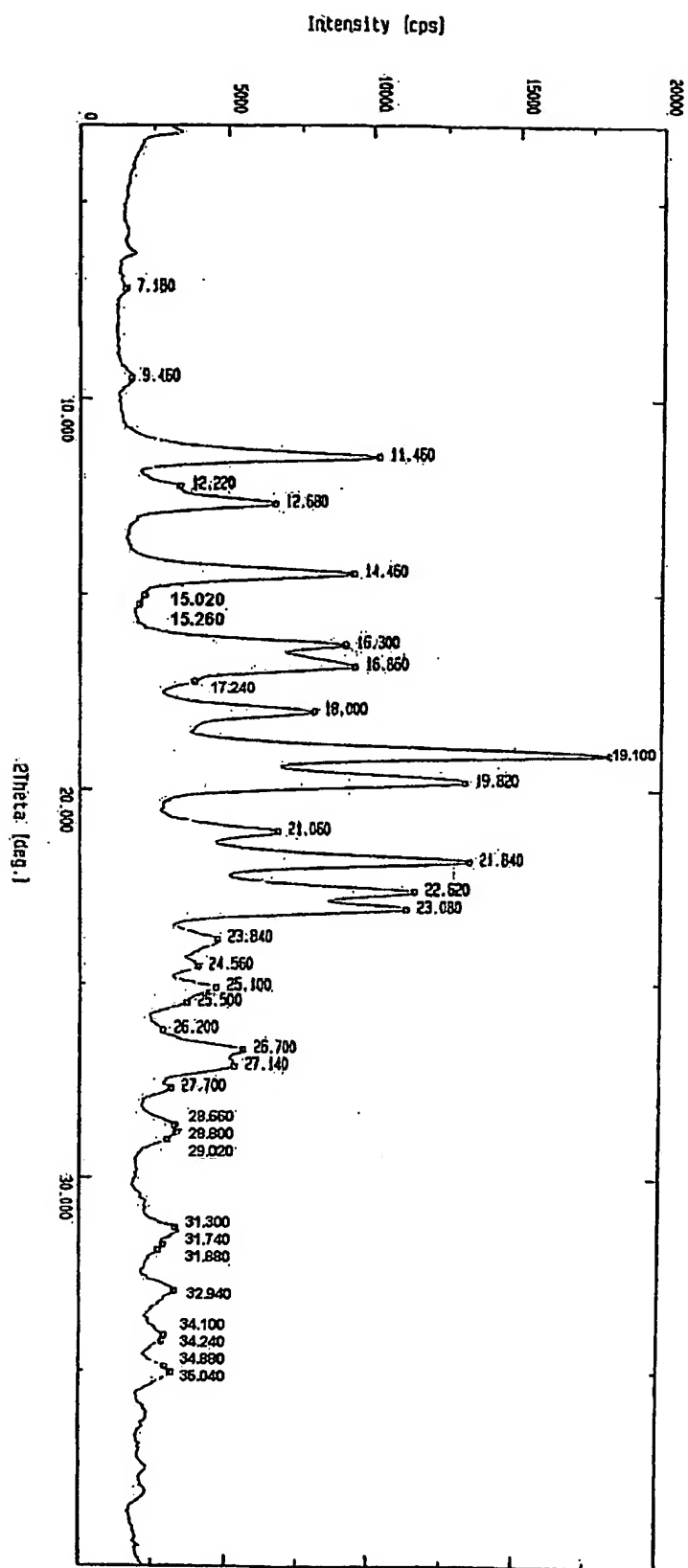
Figure 5

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C07D239/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/60804 A (ASTRAZENECA AB; ASTRAZENECA UK LIMITED; SHIONOGI & CO., LTD; TAYLOR, N) 23 August 2001 (2001-08-23) cited in the application claim 15; example 10	1-19
X	claims 1,11,15; example 9	6,7
Y	US 6 589 959 B1 (TAYLOR NIGEL P) 8 July 2003 (2003-07-08) cited in the application column 2, lines 11-40	1-19
X	EP 0 521 471 A (SHIONOGI SEIYAKU KABUSHIKI KAISHA) 7 January 1993 (1993-01-07) cited in the application page 2, lines 12-45; claim 1; examples 1,7 page 2, line 53 - page 4, line 15	1-5, 17-19

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[X] Further documents are listed in the continuation of box C.　　　　[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2005	18. 07. 05

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schuemacher, A

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/016317 A1 (TEVA PHARMACEUTICAL INDUSTRIES LTD., ISRAEL; TEVA PHARMACEUTICALS USA,) 27 February 2003 (2003-02-27) page 2, lines 15-32; claim 24; examples 7,8	16
P,A	WO 2004/014872 A1 (ASTRAZENECA UK LIMITED, UK) 19 February 2004 (2004-02-19) page 2, lines 18-30; claims 1,15	16
P,X	WO 2004/108691 A1 (ASTRAZENECA UK LIMITED, UK) 16 December 2004 (2004-12-16) page 15, lines 8-15; claim 1	1-19
E	WO 2005/021511 A1 (HETERO DRUGS LIMITED, INDIA) 10 March 2005 (2005-03-10) claims 1,6	1-19

Box II   Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 19 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III   Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of)
inventions in this international application, as follows:

    1. claims: 1-5,8-15,17

        amorphous rosuvastatin magnesium salt and the process for
        its preparation

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    2. claims: 6,7

        process for the preparation of crystalline rosuvastatin
        magnesium salt

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    3. claim: 16

        process for the preparation of rosuvastatin calcium salt

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    4. claim: 18 and 19

        pharmaceutical composition comprising rosuvastatin magnesium
        salt and its use as HMG-CoA reductase inhibitor in the
        treatment of hyperlipidemia

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB2005/000132

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0160804	A	23-08-2001	AU	775569 B2	05-08-2004
			AU	3208401 A	27-08-2001
			BG	106969 A	30-04-2003
			BR	0108378 A	11-03-2003
			CA	2397450 A1	23-08-2001
			CN	1418198 A	14-05-2003
			CZ	20022754 A3	13-11-2002
			EE	200200445 A	15-12-2003
			EP	1263739 A1	11-12-2002
			WO	0160804 A1	23-08-2001
			HU	0204051 A2	28-05-2003
			JP	2003523334 T	05-08-2003
			NO	20023853 A	14-08-2002
			NZ	520032 A	26-03-2004
			PL	356472 A1	28-06-2004
			SK	11742002 A3	04-02-2003
			US	2003045718 A1	06-03-2003
			ZA	200205331 A	03-10-2003
US 6589959	B1	08-07-2003	AT	282027 T	15-11-2004
			AU	762909 B2	10-07-2003
			AU	1882600 A	01-08-2000
			BR	9916786 A	16-10-2001
			CA	2356212 A1	20-07-2000
			CN	1333756 A	30-01-2002
			CZ	20012460 A3	17-10-2001
			DE	69921855 D1	16-12-2004
			EE	200100359 A	16-12-2002
			EP	1144389 A1	17-10-2001
			ES	2232194 T3	16-05-2005
			WO	0042024 A1	20-07-2000
			HK	1040989 A1	06-05-2005
			HU	0104828 A2	29-07-2002
			ID	29432 A	30-08-2001
			JP	2002539078 T	19-11-2002
			NO	20013368 A	05-09-2001
			NZ	512560 A	29-08-2003
			PL	348775 A1	17-06-2002
			RU	2236404 C2	20-09-2004
			SK	9632001 A3	03-12-2001
			TR	200101894 T2	21-12-2001
			US	2004009997 A1	15-01-2004
			ZA	200105187 A	23-09-2002
EP 0521471	A	07-01-1993	AT	197149 T	15-11-2000
			CA	2072945 A1	02-01-1993
			CY	2226 A	18-04-2003
			DE	69231530 D1	30-11-2000
			DE	69231530 T2	13-06-2001
			DK	521471 T3	05-02-2001
			EP	0521471 A1	07-01-1993
			ES	2153824 T3	16-03-2001
			GR	3035189 T3	30-04-2001
			HK	1011986 A1	13-07-2001
			HU	220624 B1	28-03-2002
			HU	61531 A2	28-01-1993
			JP	2648897 B2	03-09-1997
			JP	5178841 A	20-07-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB2005/000132

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0521471	A		KR	9605951 B1	06-05-1996
			LU	91042 A9	24-11-2003
			NL	300125 I1	01-07-2003
			PT	521471 T	30-04-2001
			US	RE37314 E1	07-08-2001
			US	5260440 A	09-11-1993
WO 03016317	A1	27-02-2003	US	2002099224 A1	25-07-2002
			CA	2450820 A1	27-02-2003
			CN	1543468 A	03-11-2004
			CZ	20040337 A3	12-01-2005
			EP	1425287 A1	09-06-2004
			HR	20040255 A2	31-08-2004
			JP	2005500382 T	06-01-2005
			NZ	529913 A	24-03-2005
			SK	1402004 A3	03-01-2005
			TR	200302281 T2	21-09-2004
			US	2003114685 A1	19-06-2003
			US	2004176615 A1	09-09-2004
			ZA	200309373 A	02-12-2004
WO 2004014872	A1	19-02-2004	AU	2003251369 A1	25-02-2004
			CA	2495296 A1	19-02-2004
			EP	1539711 A1	15-06-2005
WO 2004108691	A1	16-12-2004	NONE		
WO 2005021511	A1	10-03-2005	AU	2003269478 A1	16-03-2005